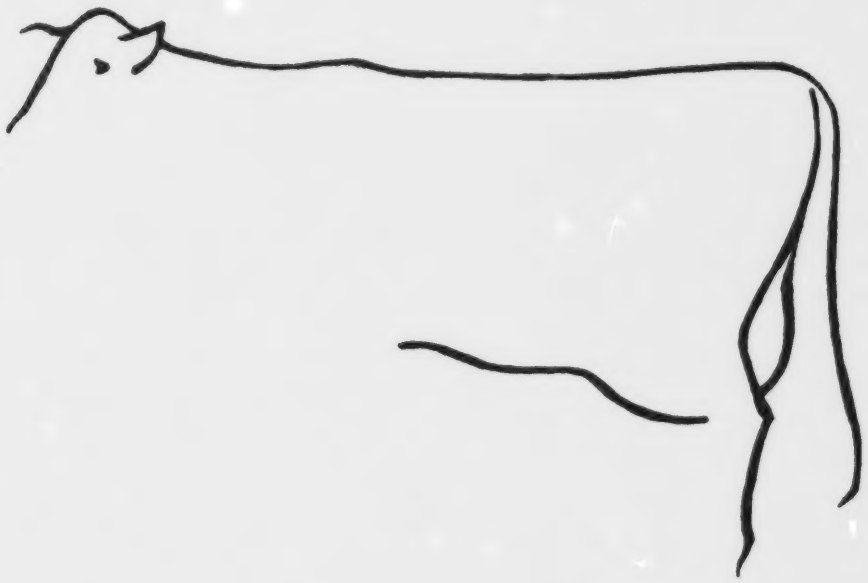
O.A.C. Publication No. 1189

Guelph Dairy Research Report



June 1989

UNIVERSITY of GUELPH

Ministry of Agriculture and Food
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GUELPH

DAIRY RESEARCH REPORT

This publication was supported by a grant from the
Ontario Milk Marketing Board.

The Dairy Research Co-ordinator wishes to acknowledge the effective editorial assistance of Ms. **Alicja Suwala** and Ms. **Heather Bailey** in the preparation of this publication.

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FOREWORD

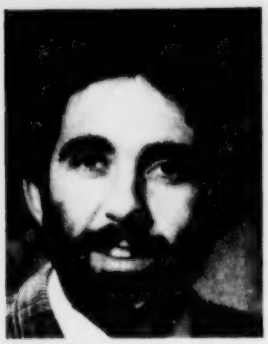
Scientific information is a prerequisite for technological advancement, maintenance of vigorous production systems and long term social prosperity. Historical experience indicates that systematic, well oriented research is one of the most productive forces necessary for such advancement. This publication summarizes accomplishments of the Dairy Research Program at the University of Guelph during the period from June 1988 to June 1989 for the purpose of extension to Ontario and Canadian dairy producers.

Activities of the Dairy Research Program, as published in this document, involve a variety of corresponding scientific disciplines and also include several multidisciplinary research projects. The principal supporter of the Guelph Dairy Research Program was the Ontario Ministry of Agriculture and Food, which provided housing facilities, animals and a major portion of the operating budget of the Elora Dairy Research Centre. Other significant contributors were Canadian Association of Animal Breeders, Natural Sciences and Engineering Research Council, Semex Canada and Ontario Milk Marketing Board. By publishing this summary the authors wish to express their sincere appreciation to the above benefactors.

D.G. Grieve, Chairman
Animal and Poultry Science

J.F.Hurnik,Coordinator
Dairy Research Program

Dairy Researchers



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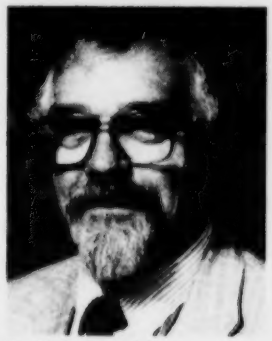
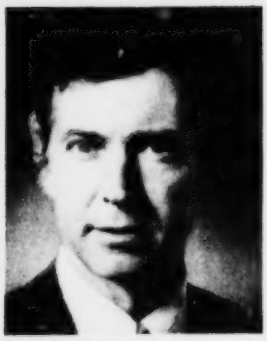
Statistics

Dr. K.J. Betteridge
Animal Biotechnology

Dr. J.G. Buchanan-Smith
Nutrition



Dr. E.B. Burnside
Breeding



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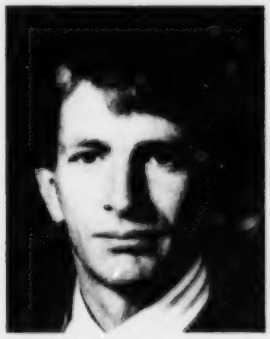
Nutrition

Dr. S.W. Etherington
Reproductive Health Mgmt.

Dr. A.M. Gibbins
Molecular Genetics



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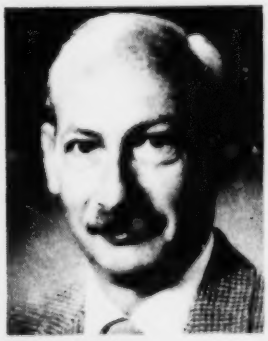
Breeding Strategies



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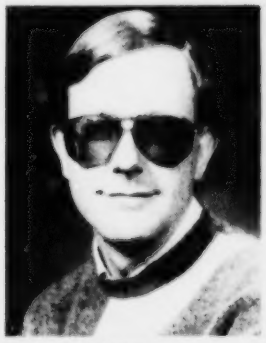
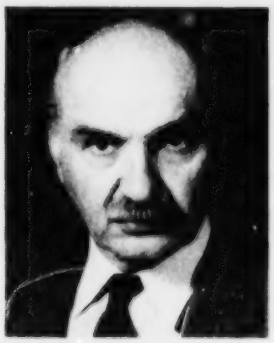
Nutrition

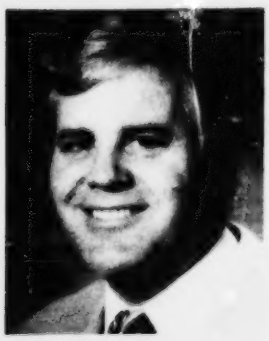
Dr. R.R. Hacker
Environmental Physiology



Dr. G. Halbert
Reproductive Health Mgmt.



Dr. J.F. Hurnik
Behaviour



Dr. B.W. Kennedy
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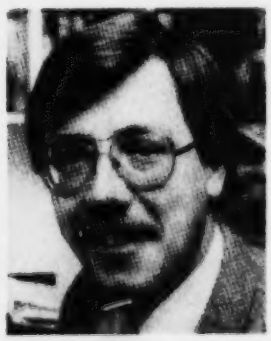
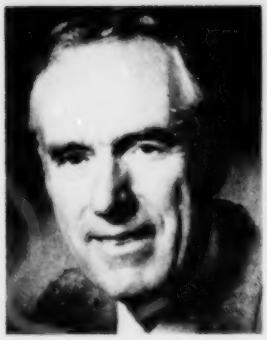
Dr. G.J. King
Reproductive Physiology



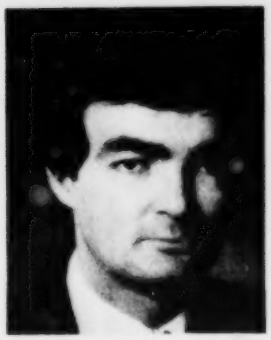
Dr. K. Leslie
Health Management

Dr. R.M. Liptrap
Reproductive Physiology

Dairy Researchers (cont'd)



Dr. G.K. Macleod
Nutrition



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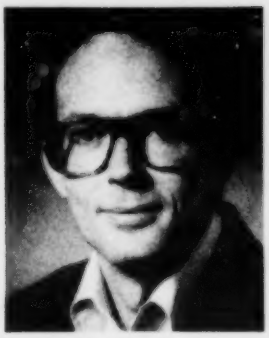
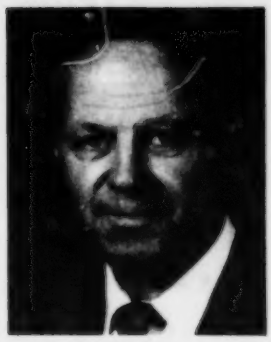
Nutritional Biochemistry



Dr. I. McMillan
Management-Statistics



Dr. L.P. Milligan
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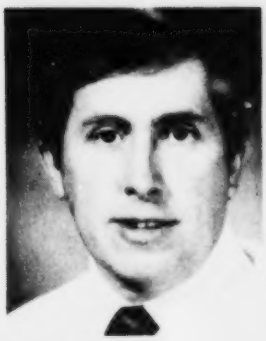
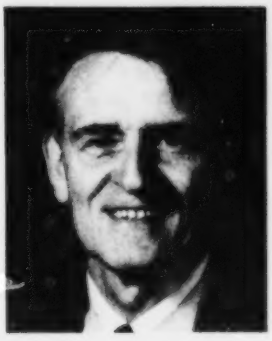
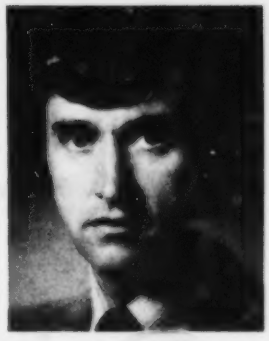


Dr. L.R. Schaeffer
Breeding



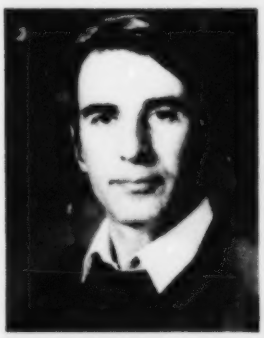
Dr. C. Smith
Genetic Improvement

Dr. R.J. Stubbings
Reproductive Biology

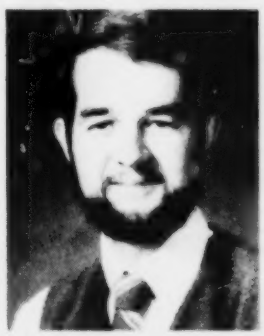


Dr. J.S. Walton
Reproductive Physiology

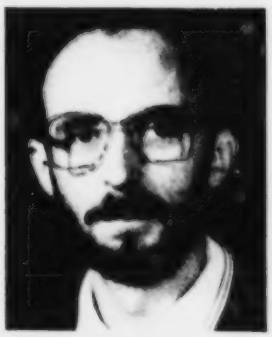
Post-Doctoral Fellowships & Research Associates



Pablo Colucci
Uruguay
cooperating faculty
— D.G. Grieve



Miguel Dominguez
Mexico
cooperating faculty
— R.J. Stubbings



Janusz Jamrozik
Poland
cooperating faculty
— L.R. Schaeffer

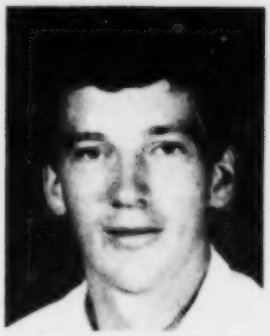
David Keller
United States
cooperating faculty
— C. Smith

Post-Docs and Research Associates (cont'd)

K. Nadarajah
Sri Lanka
cooperating faculty
— E.B. Burnside

Andrea Rapitta
Canada
cooperating faculty
— E.B. Burnside

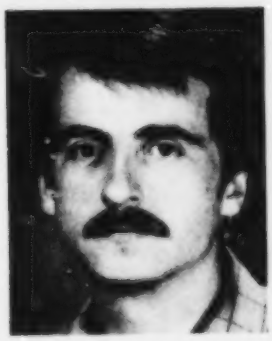
Georg Teepker
West Germany
cooperating faculty
— C. Smith



Johan Van Arendonk
The Netherlands
cooperating faculty
— C. Smith & B.W. Kennedy

Xin Zhao
China
cooperating faculty
— B.W. McBride

Data Processing & Computing Services



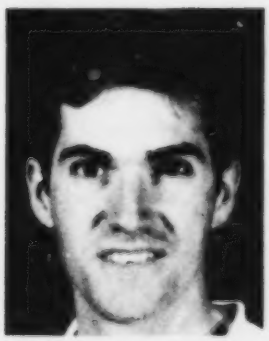
Bill Szkotnicki
Supervisor

Muriel Auld
Data Entry

Ron Lohr
Programmer Analyst

Margaret Quinton
Programmer Analyst

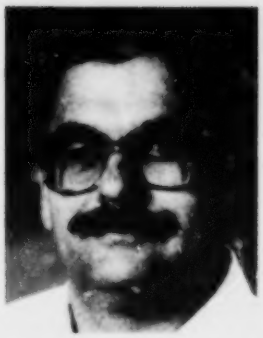
George Smith
Research Associate



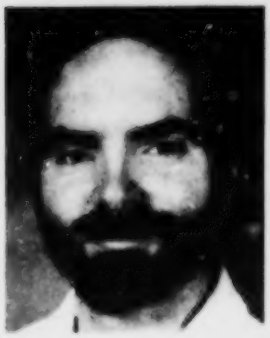
Brian Sullivan
Analyst

Muriel Tolton
Data Processing

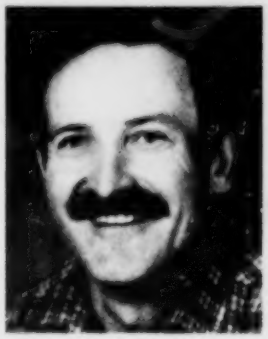
Elora Dairy Research Centre Staff



Roger Shantz
Manager



Dave Barney
Chief Technician



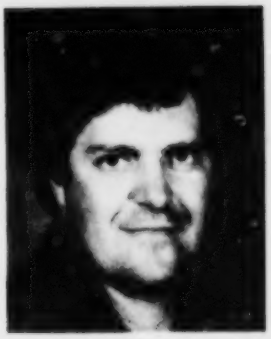
Larry Kreager
Technician

Dennis Craven
Feedsperson

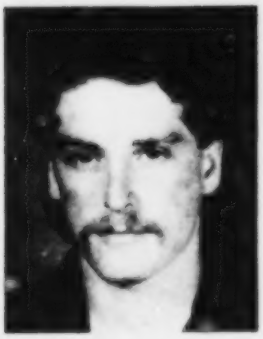
Andy Curtis
Milker I

Frank Doyle
Milker II

Ted Ferwerda
Calf Care

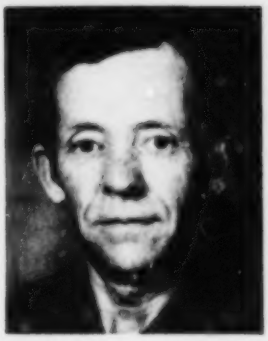


Norm Lindsay
Milker II



Mike McNabb
Herder

Peter Robertson
Calf Care



Phil Sweet
Herder

Philip Wallace
Feedsperson

GRADUATE STUDENTS

Wame Boitumelo	M.Sc.	Mike Lohuis	M.Sc.
Orville Bondoc	Ph.D.	Chris Meadows	M.Sc.
Jeanne Burton	Ph.D.	Annette Moore	M.Sc.
Nancy Campbell	M.Sc.	Robert Moore	Ph.D.
Franklin Douglas	M.Sc.	Ntombizakhe Mpofu	Ph.D.
Dan Ganesh	Ph.D.	David Muysson	M.Sc.
Leonard Golfman	Ph.D.	John Niezen	M.Sc.
Luis Gomez-Raya	Ph.D.	Ho Sung Park	Ph.D.
Nancy Graham	M.Sc.	Calvin Schalla	M.Sc.
Paul Groenewegen	M.Sc.	Kerst Stelwagen	Ph.D.
John Kelly	Ph.D.	Rob Templeman	M.Sc.
Helen Leitch	Ph.D.	Gord Vander Voort	M.Sc.
Carlos Leon-Velarde	Ph.D.	Renata Van Vliet	M.Sc.
Jicheng Liu	M.Agr.	Beatriz Villaneuva	Ph.D.

TECHNICAL SUPPORT STAFF

Lawrence Cahill
Cheryl Campbell
Janet Carrol
Jennifer Crawford
Michelle Goodwin
Diana Haluke
Terri MacKenzie
Karin Meyer
Janet O'Neill
Olwen Southwood
Kim Taylor
Linda Trouten-Radford
Helene Vaage
George Werchola

ELORA DAIRY CATTLE RESEARCH CENTRE MANAGERS' REPORT 1988/89

Roger Shantz

The past year has seen many changes here at the Research Centre. Our herd size was cut again and currently rests at 140 cows and 140 heifers, a new heifer barn was added, the manure handling system is being changed and improvements have been made to the barn itself.

The purposes of the Research Centre, however, have remained the same. Research is the main goal, with many of the results presented in this document originating from work at Elora. We co-operate with researchers from several departments as we assist them in carrying out their research. Researchers from Animal and Poultry Science, Environmental Biology and Population Medicine (OVC) make routine use of our facilities.

A second purpose is in the area of teaching. A large number of University of Guelph student labs are conducted at Elora; or animals are transported to campus for use in teaching labs. Ours is also one of the area herds used for teaching purposes with fourth year veterinary students.

A third purpose is in the area of extension. We host a large number of school tours, mainly senior high school agricultural and environmental science students. Many of these schools are in urban areas with this farm tour being a relatively new experience for the students. I believe we are able to leave an excellent impression of modern production techniques with these students. We also host many foreign visitors each year. It is always very interesting to compare notes on farming practices with these guests. Local dairy and 4-H groups are primarily interested in our research work but also show a great deal of interest in the new technology we have been able to acquire. Areas of interest include the new heifer barn, the automated milk parlour and computerized milk recording/herd management system, the feed delivery system and also our methods of handling manure. Disposal of milk wastes has also received more interest lately. Thus, while the investigation of new technologies is not one of our major purposes, we are certainly pleased to share our experiences (both good and bad) with interested dairymen.

Our production has declined over the past year and now rests at a BCA of 163 for milk, 172 for fat and 166 for protein. The main reason for this decline has been one research trial, in which we cannot cull animals for productive reasons. Milk quality continues to be good with a six month Somatic Cell Count average of 202,000 and a Plate Loop Count ranging from -3,000 to 9,000 as reported on the 'pink card' by the Dairy Inspection Branch.

Our breeding program makes use of Plus Proven sires on 75% of our herd and test sires on the remaining 25%. When selecting sires, production is emphasized. The current sires that we are using average +10.1 for milk, +12.3 for fat, and +9.4 for protein. We are also careful to watch final classification (+6.3), feet and legs (+2.1), and mammary system (+4.5). All of the bulls that we use are located in Canadian A.I. units.

A great deal of new construction has taken place over the past year. The long awaited heifer barn was completed and heifers were moved in during November of 1988. We spent time over the winter working at the final construction details in this barn. Only the installation of the Calan headgates remains to be completed. Once these headgates are in operation, we will be able to monitor individual feed consumption. This barn has the capacity to hold 78 heifers in pens equipped with Calan headgates and 76 heifers in pens with conventional self-locking headgates. In total, there are 26 pens in the barn, which allows us to group animals in a narrow age range. The small number of animals per pen also allows for excellent observation of each animal.

One of the main features of this barn is the natural ventilation system. This has been our first experience with natural ventilation and we did experience some early problems. However, we are now quite happy with this system. The centre ridge vent remains open a minimum of 5 to 6 inches year round with the side windows (which are thermostatically

controlled) opening to control the air flow through the barn. The air has always been fresh and pleasant.

The heifers have access to a bedded area and also a scrape alley which is adjacent to the feed manger. The scrape alley is cleaned three times per week. Once a week, we add shavings and cultivate the bedded area with an old stiff-tooth field cultivator. This cultivation allows the bedding pack to air out and thus extends the use we get out of each load of shavings.

A central handling area equipped with a scale/squeeze combination allows us to closely monitor the growth of these heifers. Any other research measurements can also be taken in this area.

The shed for holding the manure from the scrape alley is enclosed on three sides by cement walls. A roof was also built over this area to prevent excess rain and snow from being added to the manure. These measures allow us to prevent any potential runoff problems.

This improved facility combined with excellent quality feed and close attention to each animal has already led to an improvement in the size and stature of the heifers returning to the main barn prior to their first calving.

The main barn is now 20 years old. Many of the tie stall and free stall dividers had rusted and subsequently broken off at the cement level. We were able to replace all of these dividers this spring. The tie stall dividers were replaced with the same style as the originals. The free stall dividers were replaced with the new Model 'T' stall dividers which were designed and extensively researched in Europe. These stalls are designed to allow the cow the greatest possible comfort when in this area. One-half of the free stall dividers were cemented into the floor and the others were lag bolted onto the floor. All the stall dividers which were cemented into the floor (both tie stall and free stall) were fitted with a protective hard plastic sock which will protect the stall divider from rusting at the floor level.

A second milk waste disposal tank has also been added. All of our milk waste and also the runoff from the silo areas is collected in these tanks and then is irrigated onto the fields during the warmer months. The first tank has a capacity of 600,000 litres (130,000 gallons). This tank measures 15 metres in diameter and is 3.4 metres deep (50 feet by 11 feet). The new tank will hold 730,000 litres (160,000 gallons) and measures 16 metres in diameter and is 3.65 metres deep (53 feet by 12 feet). We believe that it is essential to do everything possible to prevent this waste from entering the water supply.

Improvements have also been made to our existing liquid manure handling system. The pipes have been moved further underground to prevent freezing. Most of the valves have also been removed from the system which should decrease the amount of service work required. The pump holes were also enlarged to accommodate the larger pumps that are now being used.

Two other projects will soon be underway. First, a new feedbarn and bedding storage area will be built to service the new barn. A new silo will also be built adjacent to this barn. Secondly, the manure system in our tie stall barn will be converted from a liquid manure to a solid manure system. The present capacity of our liquid manure system is inadequate for our current needs. Rather than expand the present liquid manure system, the decision was made to convert the tie stall area to a semi-solid manure system. Solid manure is more easily handled with the current machinery and labour supply available at the research station.

The feeding program for the Elora herd has not changed significantly. The cows continue to receive a Total Mixed Ration (TMR), while the heifers are given their feeds separately. Once the new heifer feedbarn is completed, then they too will receive a TMR. The main roughages fed are haylage and corn silage with a small amount of dry hay being offered. The grain portion of the diet consists of high moisture corn and soybean meal with a small amount of alfalfa pellets offered to the cows in early lactation. Delivery of the TMR is facilitated by the use of two computerized feed

carts. Weighbacks of refused feed have also been computerized by use of a cart designed by staff at the Dairy Centre. Complete detailed reports on feed offered and refused are available weekly to each researcher. We also have two automatic grain cars which give us the ability to feed grain many times a day.

Despite the dry spell that we experienced last summer, we were able to obtain adequate quantities of excellent quality feed (haylage, hay, corn silage and high moisture corn). An added bonus was the fact that we were able to accomplish this within the budget that we were given.

I would like to briefly mention five sources of information which we use on a regular basis, sources which have proven to be extremely valuable:

1) the preventative herd health program offered by the Ontario Veterinary College. This routine program has proven to be very beneficial. Weekly visits help us to keep up-to-date on the reproductive status of the herd. We are also able to prevent many problems before they get out of control. Thus, emergency calls are greatly reduced.

2) the ration balancing program offered by OMAF. Our head technician, Dave Barney uses this program to fine tune our rations. Certainly a large part of the increase in milk production over the past five years can be attributed to excellent quality feed which has been properly fed to our cow herd.

3) the agricultural engineering advice available through OMAF. This service has been used extensively in planning all the renovations which are listed above. The engineers continue to make themselves available to us for consultation purposes.

4) ODHIC's milk recording program. Certainly the benefits of milk recording are many. We also make extensive use of the Somatic Cell Counting service for monitoring udder health. Many problems have been corrected before they have had a chance to get a firm hold. We closely watch each cow's Somatic Cell Count and take particular interest when we see a significant increase from one test to the next. If deemed necessary, we will have the milk from all four quarters of a cow cultured to determine what type of organism is present. Thus, we are often able to determine what type of organism we are dealing with before a cow shows any clinical signs of mastitis.

5) OMMB's udder health management program. We have used this program routinely since 1984 and find it to be an excellent program. The udder health of the cows has remained at a high level and we find that this program helps point out any equipment malfunctions as well as any 'bad habits' that we might unknowingly fall into. One source of frustration has been our inability to lower our Somatic Cell Count below the 200,000 'barrier'. In January we started a program of predipping the cows' teats with an iodine solution to hopefully help us keep SCC levels below 200,000. Most of the mastitis causing organisms that we have found are environmental organisms which should be controlled by the predipping program.

As we look to the future, it is hard to predict what changes we will see. Will we see a robotic milker capable of milking cows any time of the day without any labour input? Will we see total computerization of the feed delivery system such that human labour will not be needed? Will we see a sophisticated, dynamic computerized herd management system capable of being easily adapted to <u>each</u> producer's needs? Certainly all of these are possible.

At this time, I would like to pay tribute to Ted Ferwerda, who will be retiring this August. Ted started working for the University of Guelph on February 1, 1966. The herd was located in Guelph at that time and when the move was made to Elora in 1969, Ted 'moved' with the herd. The majority of his time here at Elora was spent looking after the maternity area, calf nursery and physiology wings. His knack for working with the cow at and around the time of calving was a valuable asset. He also trained many men in the art of proper hoof care for the cow. His knowledge of hoof trimming and various hoof conditions will certainly be missed. I would like to extend a personal note of thanks for Ted's dedication to his work. I cannot help but think that any person concerned with animal care and welfare, would have his mind put at ease if he had seen the attention that Ted gave to each and every animal in his care.

ECONOMIC WEIGHTS AND OPTIMUM SELECTION OF DAIRY CATTLE WHEN MULTIPLE PRODUCTION QUOTAS APPLY

J.P. Gibson
Centre for Genetic Improvement of Livestock, Department of Animal and Poultry Science, University of Guelph

SUMMARY

The principal goal of animal breeding is maximum improvement of economic efficiency. When several traits contribute to the overall economic worth of an animal, selection can be carried out using a selection index, the principles of which are well understood. The selection index combines information on the economic value of each of the component traits with the genetic and phenotypic variances and covariances among the traits, in an optimum way for maximum improvement of economic merit.

Defining the economic weights is a critical component in the process of arriving at an optimum selection index. A method commonly used is to construct an equation, the profit equation, which describes the economic profit as a function of genetic changes in the traits of interest. The economic weights are then the partial derivatives of profit with respect to genetic changes in each of the traits. Such economic weights account not only for the increased economic returns when increasing outputs, but also the increased costs, be they management, feeding, processing or other costs.

A problem with the above approach is that no account is taken of limits on production. Such limits may be intangible, such as saturated markets, or obvious, such as quotas on production. The result of either limit is that genetic increases in output per animal must be accompanied by a reduction in the number of animals so that the overall output remains the same. This problem can be dealt with by a rescaling of the profit equation to keep output of a given trait, or overall economic output, constant. This approach has been recently applied to the problem of defining optimum selection criteria for production traits of Canadian dairy cattle (Gibson, 1988).

It is of interest to Canadian dairy cattle breeders to know how well their selection objectives match the requirements of their major export markets. Will an optimum index for Canadian cattle in Canada, also be optimum in other countries? As an example, the British dairy market was examined for comparison with results for the Canadian market (Gibson, 1988). An immediate problem was that in Britain two interdependent quotas operate on milk production. The first quota operates on milk volume, just as on fluid milk in Canada. But a second quota also operates on fat concentration, such that if the current fat concentration is exceeded, the volume quota is reduced. The method of scaling found suitable for the Canadian situation, whilst suitable for multiple independent quotas, would not deal with such multiple interdependent quotas.

Investigation of the problem revealed that with multiple interdependent quotas, there is no general algebraic definition of economic weights from a profit equation. However, for a given dependency between the quotas and a given relationship between the traits under quota and those being selected, a specific solution can be obtained. Unlike the unscaled situation, the solution depends on the response to selection. For example, if selection increases milk production but not fat concentration, the quota on fat concentration would not be triggered. The economic weights appropriate to this situation would take into account the milk volume quota only. If, however, selection increased both milk yield and fat concentration, the interaction of both quotas would have to be taken into account when deriving the economic weights. Since the response to selection is itself dependant on the economic weights, the process of defining the economic weights is iterative. In most cases there will be an unique solution, but in rare instances there may be more than one solution.

Applying the appropriate scaling for Holstein/Friesian cattle in Britain, the economic weights for liquid, fat, protein and lactose yields were found to be -1.6 p/l, 76.6 p/kg, 170.0 p/kg and 7.0 p/kg. Selection of sires based on the optimum selection index derived from these economic weights would lead to substantial rates of increase in output per cow of liquid, fat and protein, with modest increases in fat

concentration and the fat to protein ratio. Selection on a simpler index of fat plus protein yield would be more than 99% efficient and was recommended.

The expected optimum economic genetic changes in Holstein/Friesian cattle in Britain are very close to the optima derived for dairy cattle in Canada (Gibson, 1988). Thus, selection of an optimum cow in Canada should also produce an optimum cow for the British milk market, as far as milk production is concerned. In this particular example there is, therefore, no need to balance the needs of the Canadian dairy industry with those of the export markets for semen and embryos to Britain. However, this study does not take into account the widespread production of beef from the dairy herd in Britain which may well alter the balance between the need for dairy and beef characteristics. Also, there is a need to examine the requirements of other countries which currently or potentially supply a market for export of Canadian dairy cattle genetics.

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OPTIMUM NUMBER OF BULL SIRES IN DAIRY CATTLE BREEDING

M.G. Goddard and C. Smith
Centre for Genetic Improvement of Livestock
Department of Animal and Poultry Science
University of Guelph

SUMMARY

In efficient progeny testing systems, the selected elite sires, and their daughters, will be used to breed the next generation of bulls. The bull breeding part of the population is then like a dispersed nucleus breeding group, and determines the genetic response rate in the whole population. The inbreeding rate per generation is approximately $1/(8M_e)$ (M_e is the effective number of bull sires per generation), four times that in a conventional four path progeny testing system. Inbreeding leads to a reduction in genetic variance and to inbreeding depression. Methods are given to estimate the effective number of bull sires per generation to maximize the net response in economic merit, the response adjusted for these effects of inbreeding. The optimum number increases as the level of inbreeding depression, and as the number of generations considered increases. The optimum is flat especially in the use of more sires, and high proportional responses are obtained for a range of 2-3 times the optimum number. The flexibility afforded by the range may be restricted in practice, for AI breeding industry groups have to maximize their short term competitive position. For progeny testing systems, a conservative recommendation of a minimum effective number of 10 bull sires per generation for the whole breed is made, as this will allow both high genetic and high net genetic responses and will allay concern about inbreeding.

INTRODUCTION

In the past, inbreeding has not been important in most of the large dairy populations in the world, due to the number of bull sires used and to the long generation intervals. For example, in the study of Young et al. on U.S. Holsteins, the effective rate of inbreeding (measured as half the rate of increase in average relationship in the breed) was $0.19 \pm 0.08\%$ per year. So there is little cause for concern and this is reassuring in view of the dominant role world wide of North American Holstein stocks in dairy production. However, these studies have been retrospective; the Young et al. study applied to females born from 1976 to 1982. Prospective studies are needed to look at current and future trends because there are several factors which may be increasing the inbreeding rate. These include more accurate estimates of breeding value (EBV) through improved statistical methods and computing systems, greater intensification of selection and breeding on sires of outstanding merit and, through EBV's, also on the daughters of bull sires. These effects may be intensified by the use of embryo transfer and by reducing the generation interval. The objective here is to concentrate on the bull sires, and following Goddard, to develop guidelines on the number of bull sires to use per generation to maximize the net genetic response in economic merit.

PROCEDURES

In efficient progeny testing systems, there will be heavy use of sires with the highest EBV's to breed the next generation of bulls (denoted mm, males to breed males). The use of EBV's in selection of cows will lead to most of dams selected to breed young bulls being daughters of the elite (mm) bulls of the previous generation. Consequently the bull breeding part of the population is like a dispersed nucleus herd, with the same bulls used in the mm and mf paths in the nucleus section. It is the genetic change, and the inbreeding, in the nucleus section that will control the response rate, and inbreeding rate, in the whole population.

Genetic Improvement

Selection is assumed to be for the economic efficiency of production, referred to here as economic merit. The equilibrium genetic response in economic merit per generation from selection of bulls to breed bulls (mm) is

$$\Delta G = i_{mm} r_{GI} h \sigma / 2$$

where i_{mm} is the standardized selection differential, r_{GI} is the accuracy of bull selection, h^2 is the heritability and σ the standard deviation of economic merit. There will be some further response from selecting on production records among the high EBV daughters of the elite (mm) bulls of the previous generation. This can be treated as a constant in the optimization procedures considered later.

The economic value of the genetic response is reduced by inbreeding depression and the cumulative genetic response is reduced by the decrease in the genetic variation due to inbreeding. Assuming a linear inbreeding depression effect, which is reasonable at the low levels of inbreeding considered here, the net genetic response in economic merit per generation is

$$\Delta G - D\Delta F$$

where ΔF is the increase in inbreeding per generation, and D is the depression in economic merit per unit of inbreeding. The depression is often expressed as d, the percentage depression per percent of inbreeding (or the proportional depression per unit of inbreeding), so that $D = d\sigma/CV$ where σ and CV are the standard deviation and coefficient of variation of economic merit.

Over g generations, the original genetic variation (VG) will be reduced by inbreeding to $VG(1-g\Delta F)$. The net accumulated genetic response in economic merit at g generations is approximately

$$g[\Delta G(1-[\frac{g-1}{2}]\Delta F) - D\Delta F]$$

$$= g\sigma[\left[\frac{i_{mm} r_{GI} h}{2}\right](1-[\frac{g-1}{2}]\Delta F) - \frac{d}{CV}\Delta F] \qquad [1]$$

The objective here is to study the conditions which maximize the above expression, and to derive guidelines on the number of bull sires to be selected and on their usage. The problem has been studied previously for conventional progeny testing systems by many authors, originally by Robertson and Skjervold and Langholtz, and more recently by Goddard.

Inbreeding

The genetic improvement in the dispersed nucleus breeding group comes mainly from the mm path, and this allows the derivation of a simple approximate expression for the rate of inbreeding. The rate of inbreeding per generation is given by Hill as:

$$\Delta F_g = \frac{1}{32M}\left[2 + \sigma^2_{mm} + \frac{2}{k}\mathrm{Cov}_{mm,mf} + \left[\frac{1}{k}\right]^2 \sigma^2_{mf}\right]$$

$$+ \frac{1}{32F}\left[2 + k^2\sigma^2_{fm} + 2k\,\mathrm{Cov}_{fm,ff} + \sigma^2_{ff}\right]$$

where $k = F/M$, and M and F are the number of males and females entering the breeding population per generation. The term σ^2_{mm} is the variance in the number of male offspring per male (where both father and son are counted at the same age), and similarly for the other terms.

The variance terms (eg. σ^2_{mm}) derive from deliberate unequal use of bulls in the breeding population, with further random differences in the number of sons (or daughters) they actually leave which are assumed to have a Poisson distribution, with the variance equal to the intended mean for the bull concerned. Thus the number of sons per sire (n_{mm}) will have an expected value (μ_{mm}) plus a residual Poisson error (e_{mm}), and similarly for the other terms (mf, fm and ff). It is further assumed that the proportional use of a sire to breed daughters is the same as his proportional use to breed sons in the nucleus population, that is $\mu_{mf} = k\mu_{mm}$. With

$$n_{mm} = \mu_{mm} + e_{mm}$$

and similarly for the other terms, then

$$\sigma^2_{mm} = V\mu_{mm} + E(Ve_{mm}) = V\mu_{mm} + 1 \qquad \text{(Average 1 son per sire)}$$

$$Cov_{mm,mf} = kV\mu_{mm} = k(\sigma^2_{mm}-1)$$

$$\sigma^2_{mf} = V\mu_{mf} + E(Ve_{mf}) = k^2V\mu_{mm} + k$$

$$= k^2\sigma^2_{mm} - k(k-1) \qquad \text{(Average k daughters per sire)}$$

$$\sigma^2_{fm} = 1/k \qquad \text{(Average 1/k sons per dam)}$$

$$Cov_{fm,ff} = 0$$

$$\sigma^2_{ff} = 1 \qquad \text{(Average 1 daughter per dam)}$$

Substituting these terms in Hill's expression for the rate of inbreeding per generation gives

$$\Delta F_g = \frac{1}{8M}(\sigma^2_{mm} + 1/k) \qquad [2]$$

$$\simeq \frac{1}{8M}\sigma^2_{mm} \qquad \text{(since 1/k will be small)}$$

$$= \frac{1}{8M_e}$$

where M_e is the effective number of bulls to breed bulls, the number of bulls if the M_e bulls were intended to be used equally. The effect of dropping the term (1/k) will be offset by using some high EBV females which are not daughters of bull sires. Due to the equivalent proportional use of sons and daughters of mm bulls in the nucleus group, the rate of inbreeding is thus approximately four times higher than that (1/32 M_e) with a conventional large scale four-path progeny testing system. It corresponds to that in a simple two-path system with a large number of females per male.

The expression for ΔF_g can be substituted in equation [1] for net genetic response in economic merit. Order statistics are used in estimating selection differentials to allow for the limited number of sires used. With unequal usage of the mm sires, the selection differential (i_{mm}) should be weighted according to sire use. This is approximated in the expression for net genetic response by the average merit of the top M_e sires, and is a reasonable approximation.

Risk

There will be variation in the expected response in economic merit, and so some risk of obtaining less (or more) response than expected. The variance in total response over g generations

for the two path progeny test mm based system is approximately:

$$\frac{gh^2(1-r_{GI}^2)}{M_e}\sigma^2 .$$

To gauge the importance of risk, the expected response can be expressed relative to the square root of the variance. Note that the response increases as g, while its standard error increases as the square root of g.

Parameters

A range of values for the different parameters has been used to study their effects on economic response. To correspond to economic merit in which milk yield is important, a heritability of 0.25 was used. Since about half of the value of extra milk is consumed by extra costs (mainly feed costs), the coefficient of variation (CV) for economic merit (0.09) is about half of that for milk yield (0.18). Three levels of accuracy of the progeny test are used, 0.81, 0.88 and 0.93, corresponding to 30, 50 and 100 daughters. The number of bulls tested per generation (5 years) is varied from 50, representing a very numerically small breed (10 bulls tested per year), to 10,000 for a numerically large breed (eg. Holsteins world wide). No limitation has been put on the total number of females tested, and so these are mainly in the population at large. The period was varied from short term (1-2 generations), up to long term (20 generations). Three levels of inbreeding depression were used, 0.25, 0.5, 1.0 depression in economic merit per percent inbreeding. An initial attempt to quantify the economic depression (see Appendix) suggests that the low or intermediate rates may be appropriate.

RESULTS AND DISCUSSION

An objective is to outline the conditions which maximize the net genetic response in economic merit, setting the genetic gains from selection against the losses due to inbreeding. Recall that most of the genetic response and inbreeding come from bull sires in a dispersed breeding nucleus, and so it is sufficient to consider only that path, and any extra gains from selecting females can be treated as a constant. An example of the estimated net response for an intermediate set of parameter values is given in Table 1. This shows the general form of the results. For a given number of bulls tested per generation, the net response rises as the number of bull sires selected increases, but soon reaches a maximum. The fall off from the maximum is rather flat, especially for large numbers tested. So there is a rather wide range in the number selected with responses which are a high percentage of the maximum, as studied later. The number of bull sires selected giving the maximum response increases as the number tested increases along with, of course, the maximum response itself. Thus large testing schemes give large maximum net responses so it will be difficult for small schemes to compete. This result is well known and shows that schemes in small countries and in numerically small breeds are at a disadvantage. The same applies to small closed nucleus multiple ovulation and embryo transfer (MOET) schemes (11).

The effects of the other parameters on the number of bull sires selected to give maximum response are studied in turn, in Tables 2, 3 and 4. The accuracy of the progeny test has very little affect on the optimum number of bull sires (Table 2). As the number of generations in evaluation increases, the number of bull sires selected per generation increases (Table 3). Compared with a 5 generation period (25 years), the number for a 20 generation period (100 years) is increased by a factor of about 1.4 to 1.6. This reflects the accumulated loss of genetic variation with inbreeding, and its effect on reducing the genetic response. The effect of the level of inbreeding depression on the number of bull sires to maximize net response is quite large (Table 4). With a 1.0% depression of economic merit per 1% inbreeding, the number of bull sires indicated is about 2.0 to 2.5 times that with an inbreeding depression of 0.25% per 1% inbreeding.

The levels of inbreeding for the optimum schemes, those yielding the maximum net response, are quite low. With 10 bull sires per generation (5 years) used in the dispersed breeding nucleus, the estimated inbreeding per year is 1/(5(8)10) = 0.0025 or 0.25% per year, and 0.125% per year with 20 bull sires per generation. These figures can be compared to current rates in North American Holsteins of about 0.19% per year (estimated from the increase in the degree of relationship). Thus the optimization procedure is conservative, and reins back on extreme selection and use of very few bull sires. However, the rates of genetic change are appreciable. For example, the maximum net genetic response over 5 generations (25 years) with 1,000 bulls tested per generation and 12 used (Table 1) is 2.49$\pm$0.08 SD or 0.1 SD per year. This corresponds to 0.9$\pm$0.03% of the mean of economic merit (CV=0.09) per year. The standard error includes only the genetic drift variance, and not measurement error, but shows that the risk is low. Selection on production records among high pedigree EBV females will add some further response. On the other hand, use of such females as heifers for bull dams will reduce the generation interval and increase the annual rate of inbreeding.

The optimization also indicates a considerable range in the number of bull sires selected which will give high proportions of the maximum response. This is shown in Table 5, with the numbers (lower and higher) giving 95% of the maximum response. Thus there is a large range of choice (especially for higher numbers) and considerable overlap for different levels of inbreeding. For example, 10-15 bull sires per generation would give net responses greater than 95% of the maximum in all except the extreme parameter combinations in the top left and bottom right of Table 5.

Maximization of the net economic genetic response gives a rationale for the design and optimization of breeding schemes and it should be used routinely in evaluating and comparing breeding options. Note that no restriction has been put on the total number of daughters tested, so it is assumed that there is a large population of females, and that the testing requirement is small relative to the total population. With good rates of genetic trend, the predicted average EBV of the young test bulls (from the nucleus breeding group) should be competitive with those of the selected progeny tested bulls for commercial use. So the young bulls should be widely used and well tested. The value of the maximization procedure also depends on having good parameter values for the factors involved, and on including all the relevant factors in the maximization process. This is considered later.

The other critical aspect of the study has been to involve a conceptual dispersed nucleus breeding group, arising from concentration on the daughters of bull sires in breeding the next set of bulls for testing. This essentially reduces the conventional progeny testing system in dairy cattle from a four path to an approximate two path system, and simplifies the problem of maximizing the response.

The study shows that the maximization procedures achieve high rates of genetic change, with low levels of inbreeding and so are reasonable and acceptable. It also shows a flat optimum especially for numbers of bull sires larger than optimum. Thus responses which are a high proportion of the maximum are obtained with numbers 2-3 times the optimum number. This allows considerable flexibility in the number of bull sires used. It also suggests that precise parameters for inbreeding depression of performance may not be critical.

There may be effects other than inbreeding which should be taken into account. One is the effect of previous selection of parents on the genetic variation among bulls which are available for selection. This has been shown to be appreciable in progeny testing in dairy cattle; Meyer and Smith found that a simulated heritability of 0.25 was reduced to 0.19. However, most of the estimates of the heritability of milk yield have been calculated from sons of selected sires, so the reduced genetic variance for selection may already be largely accounted for. Another factor reducing response can be the high correlation in EBV's for family members, leading to selection differentials lower than predicted. This is not likely to be important in selection of bulls, since most of the information on bulls will come from an accurate independent progeny test. There may be other factors, such as inefficient selection, selection on non-economic traits or unknown factors, reducing the response so it might be argued that it would be wise to adopt a conservative policy and use more bull sires than the optimum number,

since large proportional responses are still obtained.

The result showing 10-15 bull sires per generation (5 years) corresponds to the recommendation of 2-3 bull sires (n_B) per batch by Skjervold and Langholtz. This is surprising since the inbreeding rate is four times as high with the dispersed nucleus system. Here the expression maximized is of the form (with k_i approximately constant)

$$\left[\frac{i_{mm} k_1}{2} - \frac{k_2}{8M_e} \right]$$

while they seem to have used (incorrectly), with $M_e = 5n_B$

$$\left[i_{mm} k_1 - \frac{k_2}{32n_B} \right] - 2 \left[\frac{i_{mm} k_1}{2} - \frac{k_2}{12.8M_e} \right]$$

instead of

$$\left[\frac{i_{mm} k_1}{2} - \frac{k_2}{32M_e} \right].$$

So the expression they used and the results derived are similar to those in the current study. Thus they seem to have overestimated the number of bull sires per generation to give maximum response in a four path system but their long established recommendation of 2-3 bull sires per batch (10-15 per generation) seems appropriate for the future two path systems. Note that the recommendations here are in terms of effective numbers (M_e) of bull sires per generation assuming equal use. In practice there will be heavier use of the better bull sires. With σ^2_{mm} the variance in sons per sire, the total number of bull sires used will be $M = M_e \sigma^2_{mm}$. If bull sires are used over 3-5 years the number in use at any one time will correspond to the number per generation.

There is likely to be a conflict between the long term national interest in maximizing net response, and the short term competitive interests of AI breeding groups. Bull studs have some incentive to use several bull sires because of market demand for bulls which are unrelated to the cow population and to other bulls, and adjustments may be made for this. There may also be an aversion to risk and an attempt to increase genetic variance within young bull teams which leads them to use more than the minimum number of bull sires. However, to stay in business, and contribute to further improvement, breeding groups must be chiefly concerned with their short term competitive position. This depends on finding bulls of outstanding merit. Their main strategy must be, like all their competitors, to breed from the very best bull sires (and bull dams) to increase their chance of getting a top-ranking bull in the next generation, and they will be less concerned with future inbreeding in the population. This may lead to a decrease in the effective number of bull sires used. Thus in practice it may be the net response at sub-optimal numbers of bull sires that is important, and the fall off in net response is much greater as the number decreases. The situation is illustrated in Figure 1, showing the net response for different levels of inbreeding depression (0.0, 0.25 and 1.0%/%). Inbreeding decreases rapidly as more bull sires are used, and the net responses take on a similar trend. A balance between high short term genetic gains, ignoring future inbreeding, and the long term net genetic responses is achieved with a comparatively low effective number of bull sires per generation. A conservative minimum of about 10 bull sires per generation may be recommended, as allowing high short term gains while allaying fears about inbreeding, and its long term consequences.

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Appendix Estimation of change in economic efficiency per 1% inbreeding

Economic efficiency[5]

Products - per cow per year	
Milk sales 7022 kg x 0.279	$1959
Other sales (male calves, surplus heifers, cull cows)	354
	$2313

Input	
Rearing costs	
Feed and nonfeed	$332
Production costs	
Total nonfeed costs	1236
Feed costs	
Maintenance and pregnancy	313
Feed for milk	395
	$2276

Economic efficiency = (Value of outputs/Cost of inputs)
= 2313/2276 = 1.0163

Inbreeding effects per 1% inbreeding

Milk yield -22 kg[3,4,7]
Mortality (x1.02)[7]
Health costs (x1.02) Estimated from 7
Calving interval 0.12 days[4]
Female fertility (x0.997) Estimated from 4
Male fertility (x0.997) Taken as same as for females. But see 2.

Cow, cost statistics

Milk yield = 7022 kg[5] Value = $0.279 per kg[5]
Mortality (adult equivalent) = 0.05
Fertility per mating = 0.5 Semen cost per mating = $15[1]
Health cost = $37[6]

Changes in output value and costs per 1% inbreeding

<u>Changes in output value</u>		
Less milk per cow	(-22)(0.279)	-$6.1
Higher mortality		
Loss in animal sales	(0.05)(0.02)(354)	- 0.4
Loss in milk sales (half period)	(0.05)(0.02)(0.5)(7022)(0.279)	- 1.0
Lower fertility (male and female)		
Loss in milk sales	2(7022)(0.279)(0.12)/365	- 1.3
		-$8.8
<u>Changes in costs</u>		
Feed saved from less milk	-0.5(6.1+1.0+1.3)	-$4.2
Higher health costs	37(0.02)	+ 0.7
Extra semen	2(0.003)(2)(15)	+ 0.2
Saving in maintenance costs (deaths, half period)	(0.05)(0.02)(0.5)(313)	- 0.2
		-$3.5

Change in economic efficiency per 1% inbreeding

$$-\left[\frac{2313-8.8}{2276-3.5} - 1.0163\right] 100\% = -0.24\%$$

1 Beaudry et al., 1988.
2 Flade and Zeller, 1976.
3 Hodges et al., 1979.
4 Hudson and Van Vleck, 1984.
5 Keller, 1988.
6 Shanks et al., 1982.
7 Young, 1984.

Table 1. Net genetic response in economic merit (SD units) over five generations of progeny testing and selection[1].

Number (M) of bulls to breed bulls per generation	Number (T) of bulls tested per generation							
	50	100	200	500	1000	2000	5000	10000
2	0.23	0.49	0.73	1.02	1.23	1.42	1.67	1.84
4	0.98	1.29	1.57	1.90	2.13[2]	2.35	2.62	2.81
6	1.13	1.47	1.77	2.12	2.37	2.60	2.88	3.08
8	1.15	1.51	1.82	2.20	2.45	2.69	2.98	3.19
10	1.12	1.50	1.83	2.22	2.48	2.73	3.03	3.24
12	1.07	1.48	1.82	2.22	2.49	2.74	3.04	3.26
14	1.03	1.44	1.80	2.21	2.48	2.73	3.05	3.27
16	0.97	1.41	1.77	2.19	2.47	2.73	3.04	3.27
18	0.91	1.37	1.74	2.17	2.45	2.72	3.03	3.26
20	0.86	1.33	1.71	2.15	2.44	2.70	3.02	3.25
22	0.80	1.29	1.68	2.12	2.42	2.69	3.01	3.24
24	0.74	1.25	1.65	2.10	2.40	2.67	3.00	3.23
26	0.69	1.21	1.62	2.08	2.38	2.65	2.98	3.22
28	0.63	1.17	1.59	2.06	2.36	2.64	2.97	3.21
30	0.57	1.13	1.56	2.03	2.34	2.62	2.96	3.20

[1] Accuracy of progeny test 0.877; 50 effective daughters. Inbreeding depression in economic merit, 0.5% per 1% inbreeding.

[2] Expression (1)

$$= 5\left[\frac{2.92(0.877)(0.5)}{2}\left[1-\frac{4}{2}\frac{1}{(8)4}\right]-\frac{0.5}{0.09}\frac{1}{8(4)}\right] = 2.13.$$

Table 2. Effect of accuracy of bull progeny test on the number of bulls to breed bulls to maximize net genetic response in economic merit[1].

Number (T) of bulls tested per generation	Accuracy of progeny test		
	0.81	0.88	0.93
50	8	8	7
100	9	9	8
200	11	10	9
500	11	11	10
1000	13	12	11
2000	13	13	13
5000	15	15	14
10000	16	15	15

[1] Responses evaluated over five generations. Inbreeding depression in economic merit, 0.5% per 1% inbreeding.

Table 3. Effect of the number of generations in evaluation on the number of bulls to breed bulls to maximize net genetic response in economic merit[1].

Number of bulls tested per generation	Number of generations				
	1	2	5	10	20
50	7	7	8	8	10
100	8	8	9	10	12
200	9	9	10	11	14
500	10	10	11	13	17
1000	10	11	12	15	17
2000	11	11	13	16	21
5000	11	13	15	17	23
10000	14	14	15	20	23

[1] Accuracy of progeny test 0.88; 50 effective daughters. Inbreeding depression in economic merit, 0.5% per 1% inbreeding.

Table 4. Effect of inbreeding depression on the number of bulls to breed bulls to maximize net genetic response in economic merit[1].

Number of bulls tested per generation	Inbreeding depression in economic merit (%/%)		
	0.25	0.5	1.0
50	5	8	12
100	6	9	14
200	6	10	16
500	7	11	18
1000	8	12	20
2000	8	13	21
5000	10	15	23
10000	10	15	26

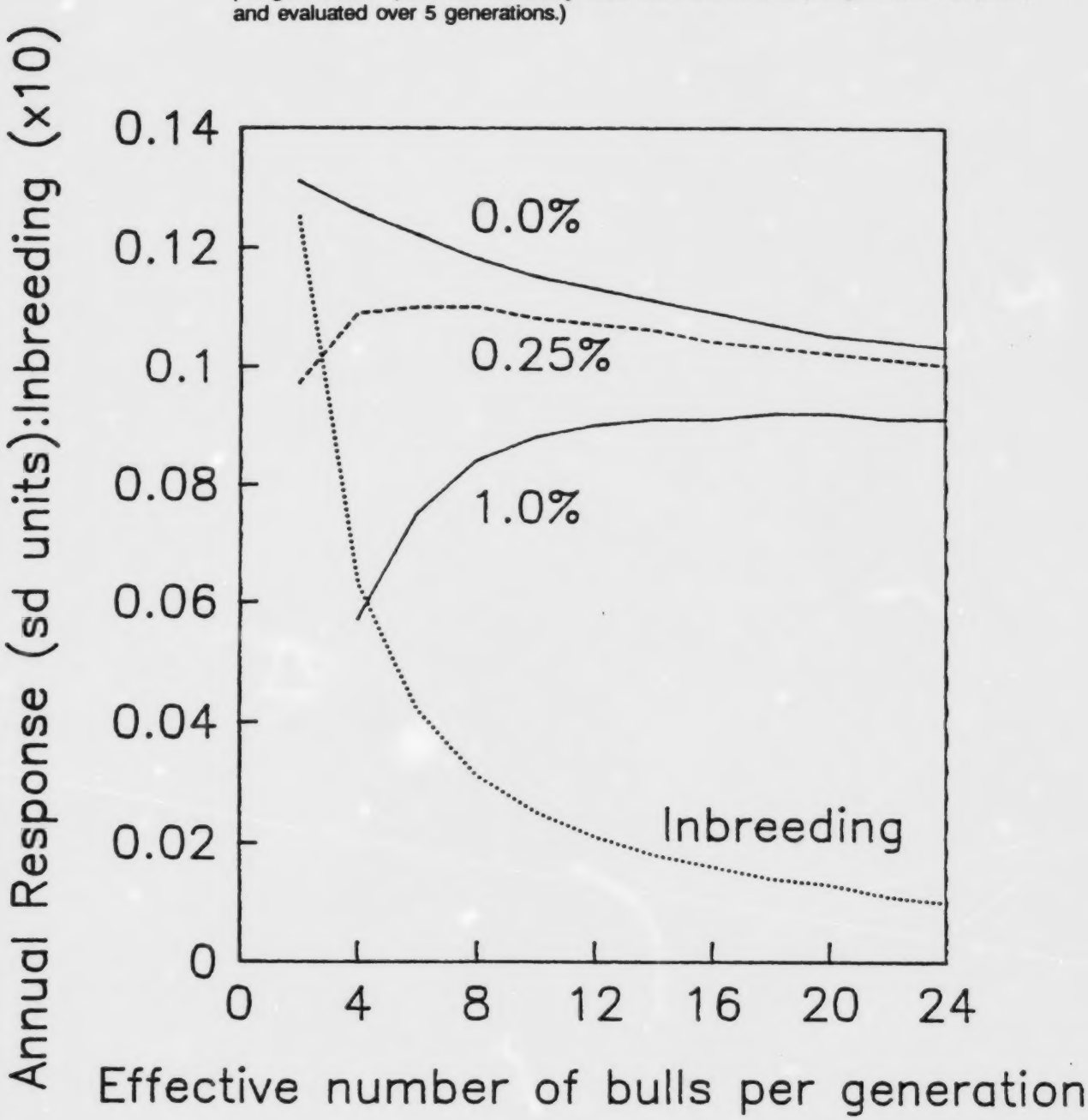
[1] Accuracy of progeny test 0.88; 50 effective daughters. Responses evaluated over five generations.

Table 5. Number (M) of bull sires selected per generation to give maximum net response, and lower (L) and higher (H) numbers giving 95 percent of the maximum.

Number of bulls tested per generation (T)	Number of generations (g)		Inbreeding depression (%/%) 0.25	0.5	1.0
50	5	L	4	6	9
		M	5	8	12
		H	8	11	16
	20	L	6	8	11
		M	8	10	14
		H	11	14	18
200	5	L	4	6	10
		M	6	10	16
		H	12	18	27
	20	L	7	9	13
		M	11	14	21
		H	19	24	32
1000	5	L	4	6	11
		M	8	12	20
		H	19	27	42
	20	L	8	10	15
		M	15	17	26
		H	33	40	52
10000	5	L	4	7	11
		M	10	15	26
		H	31	45	71
	20	L	9	12	16
		M	21	23	37
		H	59	71	94

Accuracy of progeny test = 0.88; 50 effective daughters.

Figure 1. Inbreeding and the net genetic response with inbreeding depression (d) of 0.0%, 0.25% and 1.0% per 1% inbreeding for different effective numbers of bull sires used per generation. (Selection accuracy 0.88, 1000 bulls tested per generation (5 years), and evaluated over 5 generations.)



ALTERNATIVE MATING POLICIES FOR CONSECUTIVE SUPEROVULATIONS OF DONOR COWS

D.S. Keller and C. Smith
Department of Animal and Poultry Science, University of Guelph

SUMMARY

Donor cows selected for superovulation (flush) are often subject to more than one flush. One or more sires can thus be used per donor. The effects of mating donor dams to one or more sires on the accuracy of evaluating individual bulls and full-sib bull groups (bull teams) was examined. Twenty donor dams were assumed to be flushed 4 times and mating options were: 1) all flushes/cow mated to same sire; 2) 2 flushes/cow mated to one sire, 2 flushes mated to an alternative selected sire; and 3) each flush/cow mated to a different selected sire. The number of offspring per donor was set to 2, 4, 6, or 8. Bulls were evaluated at 3 years of age (based on records on paternal and maternal half-sisters, and full sib sisters), and at 5 years of age (20, 50 or 100 daughter records per bull added to index). Mating policies had essentially no effect on the accuracy of evaluating individual bulls because the amount of information available is unaffected. The accuracy of bull team selection was reduced by using mating policies 2 or 3 rather than 1. This was especially true for 3 yr evaluations. Assuming 4 offspring per donor, selection accuracies were .71, .63, and .48 for mating policies 1 to 3, respectively. These results arose because the size of the bull team is reduced by half (mating policy 2) or fourfold (mating policy 3) relative to mating policy 1. The loss in accuracy of bull team evaluation with mating policies 2 and 3 is compensated for by the increased selection intensity possible among the more numerous smaller teams. For an example assuming 2 bull teams are to be selected at 5 yrs, the net genetic merit of selected teams (accuracy*selection intensity) using mating policies 2 and 3 were up to 18 and 30% greater than using mating policy 1. The relative advantages of policies 2 and 3 are larger with less intense bull team selection. Results for selection at 3 yrs showed that mating policy 2 was superior to mating policy 1 for all offspring numbers per donor but mating policy 3 was inferior to mating policy 1 for offspring numbers per donor of less than 8. In sum, using more than 1 sire per donor cow on consecutive flushes has no effect on the accuracy of evaluating individual bulls but can lead to higher levels of genetic merit for bull team selection by increasing selection intensity at the expense of reduced selection accuracy.

THE EFFECT OF VARIABILITY IN RESPONSE TO SUPEROVULATION ON DONOR COW SELECTION DIFFERENTIALS IN NUCLEUS BREEDING SCHEMES

D.S. Keller and G. Teepker[1]
Centre for Genetic Improvement of Livestock, Department of Animal and Poultry Science, University of Guelph and [1]Institute for Animal Breeding and Genetics, University of Goettingen

SUMMARY

The effectiveness of nucleus breeding schemes based on multiple ovulation and embryo transfer in practice is highly dependent upon the achieved reproductive rate. This study quantifies the effects of donor cow variability in response to superovulation (random and phenotypically correlated with milk yield) and failure rates (proportion of selected cows producing 0 transferable embryos) on the mean and variance of realized female selection differentials as affected by herd size, average embryo yield, and the coefficient of variation for embryo yield. Results show that differences in failure rates (0 to 40%) caused reductions of up to 64% in realized female selection differentials. The existence of a negative phenotypic correlation (-.3) between donor cow embryo yield and milk yield caused smaller reductions (up to 19%). Variability in realized female selection differentials is largest for small nucleus units and should not be neglected when planning the establishment of a nucleus breeding unit. The use of multiple flushes rather than a single flush per cow to produce the same average number of transferable embryos can reduce the variability in realized selection differentials but increases generation interval. Variability in response to superovulation will also affect inbreeding rates and the average accuracy of selection. Simulation studies are needed to quantify the total effect of variability in response to superovulation on the genetic responses possible in nucleus breeding schemes relying on multiple ovulation and embryo transfer.

INTRODUCTION

Theoretical and simulation studies of nucleus herd breeding schemes for dairy cattle utilizing multiple ovulation and embryo transfer (MOET) have to date assumed a constant level of embryo production from donor cows (9,13,14,16) In practice embryo yields from superovulated dairy cows are quite variable (4), and a fraction of cows fail to produce transferable embryos in any one flush (12). Additional cows of inferior genetic merit must be selected to replace those donor cows that fail. The resulting loss of genetic merit is expected to be small with large numbers of potential donors available. However, proposed MOET schemes often have small numbers of potential donors and the effect of donor cow failure on realized selection differentials for these schemes needs to be quantified. It is yet to be demonstrated whether or not embryo yield following superovulation is correlated with milk yield level. Other reproductive measures have generally been found to be negatively phenotypically correlated with milk yield of dairy cows (5). This study will examine the effect of failure rates and phenotypic correlations between embryo yield and milk yield on the mean and variance of realized female selection differentials as affected by herd size, average embryo yield and the coefficient of variation for embryo yield.

PROCEDURES

Donor cows for superovulation are assumed to be selected from a nucleus herd with 64, 256 or 1024 cows. Phenotypic values for the trait of economic importance (e.g. milk yield) expressed as deviations (in phenotypic standard deviation units) from herd mean were assigned to cows in descending rank order based on expected selection differentials in finite populations (1). The average number of transferable embryos (μ) per cow is set at 8, or 16 embryos which are high currently. These can be produced either in one single flush with 8, or 16 embryos, or with 1, or 2 flushes yielding 8 embryos at a time. A proportion f (f= .0 to .4) of all cows is randomly assigned to produce no transferable embryos (called failure rate). If multiple flushes are used, success or failure on a subsequent flush is assumed to be independent of results from the previous flush. Assuming milk yield and embryo yield among responding cows have a bivariate normal distribution, a cow's embryo yield conditioned

on her phenotypic milk yield value was calculated (3) as

$$E_i = \mu + r_p * \sigma * MY_i + DEV_i$$

where, E_i = number of transferable embryos for cow i; μ = average number of transferable embryos per cow (8, or 16); σ = standard deviation of the number of transferable embryos per cow; $\sigma = \mu$*CV, where CV is the coefficient of variation in embryo yield among responding cows (20 or 50%); r_p = phenotypic correlation between number of transferable embryos and milk yield; MY_i = phenotypic value for milk yield for cow i expressed as a deviation from herd average in σ units; DEV_i = random deviation of number of transferable embryos for cow i = $Z * \{(1 - r_p^2)*\sigma^2\}^{1/2}$, with Z being a random number from a standard normal distribution.

Literature estimates of the CV for embryo yield following superovulation have been near 100% when non-responding cows are included (4,17). To be conservative, the CV for embryo yield among responding cows only was set at 20 or 50%. The number of recipients that are required for embryo transfer was set at four times the herd size, since it is assumed that four embryo transfers result in one female offspring (50% survival rate, sex ratio 1:1) to replace a cow in the nucleus herd. Selection differentials, weighted by the number of transferable embryos, were calculated by selecting cows with the highest milk yield until the total number of transfers required was reached. The average and standard deviation of realized selection differentials were computed from 1000 replications for each combination of background variables. There was no restriction on the selection of related females and no adjustments of selection differentials for population structure (6) were used.

RESULTS

Average realized female selection differentials with accompanying standard deviations over 1000 replicates are shown in Table 1 for alternative failure rates (f), herd sizes, average embryo yields (E) per cow (single flush), and a CV for embryo yield equal to .2 or .5. Expected large sample selection differentials with f=.0 for E of 8 and 16 are .798 (50% selected) and 1.271 (25% selected), respectively. Results in Table 1 with f=.0 show the effects of random variability in embryo yield. Average selection differentials are lower in small herds because of finite sampling adjustments (1). Trends with herd size were more pronounced with highly variable embryo yields (CV=.5). Standard deviations of realized selection differentials were largest in small herds and were approximately halved for each fourfold increase in herd size. Standard deviations were more than twice as large with CV=.5 as they were with CV=.2. Using multiple flushes rather than a single flush to produce the same average embryo yield per donor reduces variation in realized selection differentials by from 26 to 50% (not shown). For each herd size and embryo yield realized female selection differentials decrease substantially as f increases. The effect of f is strongest for low embryo yields. Increasing f also causes large increases in the standard deviation of realized female selection differentials.

The effects of f and a phenotypic correlation (r_p) between embryo yield and milk yield on average realized selection differentials is shown in Table 2 for a herd size of 256. Percentage reductions in realized selection differentials due to f and r_p were found to similar for all herd sizes examined. Results in Table 2 show that reductions in realized selection differentials from f alone (r_p=.0) are unaffected by the CV for embryo yield. The effect of f was more than twice as large at low selection intensity (50% for E=8) as they were at higher selection intensity (25% for E=16). Results in Table 2 show that the effect of r_p on realized selection differentials was larger with higher CV as expected from [1] but was always much less than the effect of f. Results in Table 2 also show that the reductions in realized selection differentials due to r_p was essentially independent of f (i.e. additive effects). This outcome arises from the model used and may not precisely reflect the true biological system. The combined effects of f and r_p on realized female selection differentials can be dramatic. The worst case scenario (f=.4 and r_p=-.3, CV=.5) yields only 18 or 61% of the differential possible for average embryo yields of 8 and 16, respectively.

The effects of f and r_p on realized female selection differentials detailed above arise because they each cause increases in the total number of cows that must be superovulated to produce the required number of transferable embryos. These factors will thus prove costly financially as well as genetically. An example of the effects of f and r_p on the total number of donor cows that have to be superovulated is shown in Table 3. For the case illustrated the number of cows superovulated assuming a constant embryo yield is 64. Results in Table 3 show that with f=.0 and r_p=.0 the average total number of cows superovulated was 64. Increasing f and a negative rp are shown to increase the total number of cows superovulated. Results show that for the worst case scenario (f=.4 and r_p=-.3) nearly twice as many cows must be superovulated as assumed ignoring these effects. Unless superovulation techniques become inexpensive these results identify what could be a major operational concern for MOET based breeding systems. Also shown in Table 3 are the numbers of cows actually contributing embryos. Increasing f had no effect on the number contributing using a single flush but increased the number contributing using multiple flushes. Imposition of a negative r_p increased numbers of cows contributing embryos in all cases. Assuming consecutive flush outcomes are independent, the use of multiple flushes as opposed to a single flush to achieve the same average embryo yield per donor increases the number of cows that contribute embryos.

DISCUSSION

The effectiveness of MOET based breeding schemes in practice is highly dependent upon the achieved reproductive rate. The majority of theoretical and simulation studies have so far ignored the effect of variability in response to superovulation on response to selection in MOET breeding schemes. Jansen and Schlotte (8) found using simulation that variability in family size had only small effects on selection response. These authors, however, compared variable family size results to constant family size results with a lower average embryo yield per donor. The expected selection intensities and, therefore, selection differentials were the same for the two systems. Results of this study show that variation in embryo yields from donor cows can have substantial effects on the mean and variance of realized female selection differentials. Although our emphasis has been on dairy cattle MOET breeding schemes, results can be generalized within the range of assumed parameters to breeding plans in any species utilizing MOET. The results show that increasing failure rates caused reductions of up to 64% in realized female selection differentials. The existence of a negative phenotypic correlation between embryo yield and milk yield caused smaller reductions (up to 19%). The maximum failure rate used in this study was 40%, whereas Monniaux et al. (12) have reported failure rates of 25 to 52% for superovulated Holstein cows. The effects of failure rates on realized female selection differentials reported in this study are therefore not exaggerated. No comparative values for the phenotypic correlation between milk yield and embryo yield of donor cows were available from the literature. If a negative genetic correlation between milk yield and embryo yield exists it will likely be somewhat reduced by selection over time if family sizes are not standardized.

Our results are for small to moderately sized closed nucleus breeding schemes. McGuirk (11) has noted that for larger schemes with high reproductive rates the effect of variability in response to superovulation on realized female selection differentials will likely be small. For hybrid breeding schemes combining nucleus breeding units with conventional progeny testing programs (15) or open nucleus schemes (2), the population of potential donor cows is very large and the effects of variable embryo yields are likely to be negligible. Thus, variability in response to superovulation will make closed nucleus breeding schemes less competitive with alternative breeding schemes.

Our results also show that variability in response to superovulation leads to variability in realized female selection differentials. Variability in realized female selection differentials is largest in small nucleus units. Variability in realized selection differentials and therefore response should be considered when planning to establish a nucleus breeding unit.

The use of multiple flushes rather than a single flush per cow to produce the same average number of transferable embryos can reduce the variability in realized female selection differentials but will increase the generation interval.

This study has only addressed the effect of variability in response to superovulation on realized female selection differentials. Variability in embryo yields will also affect inbreeding rates and the accuracy of selection. The total effect of variability in response to superovulation on the effectiveness of MOET based breeding schemes is likely to be sizable and needs to be estimated with simulation. Realized inbreeding rates will be determined by the effective population size which increases with the number of cows contributing progeny but decreases with variable family size (6). It can be shown that average accuracy of selection is expected to decrease with random variation in family size. A negative correlation between embryo yield and the selection trait will further reduce the average accuracy of selection because highest performing animals are likely to have smaller than average family size.

It appears there are strong incentives for the use of standardized family sizes in MOET based breeding schemes. For a fixed nucleus size and mating policy there will be an optimal embryo yield per donor determined by the tradeoff between selection intensity, inbreeding rates and loss of genetic variance from selection (10). Perhaps superovulation and embryo recovery methods can be improved so that the number of embryos per cow can be predetermined. With current technology one could plan to flush cows repeatedly until the desired numer of embryos is reached. This, however, would lead to increases in the generation interval. In any case, even with a constant level of embryo production, variability in embryo viability and offspring survival rates are remaining sources of variation in family size.

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Table 1: Average realized female selection differentials and accompanying standard deviation over 1000 replications as affected by failure rate (f), herd size, average embryo yield (E), and the coefficient of variation for embryo yield (CV).[1]

		CV=.20 Herd size			CV=.50 Herd size		
E	f	64	256	1024	64	256	1024
8	.0	.786 (.036)	.796 (.019)	.798 (.010)	.782 (.086)	.800 (.045)	.801 (.024)
	.2	.594 (.097)	.605 (.048)	.607 (.029)	.593 (.131)	.609 (.066)	.610 (.038)
	.4	.268 (.175)	.291 (.093)	.299 (.047)	.269 (.202)	.295 (.110)	.304 (.057)
16	.0	1.250 (.038)	1.267 (.019)	1.270 (.011)	1.245 (.091)	1.269 (.047)	1.273 (.026)
	.2	1.112 (.098)	1.129 (.049)	1.132 (.028)	1.109 (.133)	1.131 (.068)	1.133 (.038)
	.4	.903 (.156)	.928 (.079)	.935 (.043)	.897 (.188)	.931 (.094)	.937 (.052)

[1] Values computed with phenotypic correlation set to 0.

Table 2. Relative effects of failure rates (f) and a phenotypic correlation (r_p) between embryo yield and milk yield on average realized female selection differentials.[1]

		Failure rate								
		.0			.2			.4		
		Phenotypic correlation								
CV	EY	-.3	.0	.1	-.3	.0	.1	-.3	.0	.1
.20	8	92	100	102	68	76	79	29	36	39
	16	95	100	101	84	89	91	68	73	75
.50	8	81	100	106	57	76	82	18	37	43
	16	88	100	104	77	89	93	61	73	77

1 Average realized selection differentials (herd size=256, single flush, 1000 replications) with f=.0 and r_p=.0 set to 100 in each case.

Table 3. Effects of failure rates and a phenotypic correlation between embryo yield and milk yield on the number of donor cows superovulated and those contributing embryos.[1]

	Failure rate					
	.0		.2		.4	
	Phenotypic correlation					
Number of cows	-.3	.0	-.3	.0	-.3	.0
Total	77	64	94	80	122	107
Contributing Embryos						
Single flush	74	63	72	63	70	63
Multiple flush[2]	77	64	89	77	100	88

1 Herd size=256, average embryo yield=16, CV=.5.

2 Multiple flush signifies that 2 flushes averaging 8 embryos per flush were used. Embryo yield on second flush was assumed independent of first flush outcome.

GENETIC EVALUATION UNDER AN ANIMAL MODEL WHEN IDENTICAL GENOTYPES ARE REPRESENTED IN THE POPULATION

B.W. Kennedy and L.R. Schaeffer
Centre for Genetic Improvement of Livestock
Department of Animal & Poultry Science
University of Guelph

SUMMARY

Use of split embryos and other forms of cloning as well as the natural occurrence of monozygotic twins results in animals in the population that are genetically identical. This can complicate genetic evaluation under an animal model because the matrix of additive genetic relationships among animals, **A**, is singular and $\mathbf{A}^{-1}$ does not exist. Solution of mixed model equations with singular **A** is computationally difficult and is feasible only for small populations. It is shown algebraically and illustrated through numerical example that defining **A** as the matrix of additive genetic relationships among genotypes, rather than animals, and treating records on clones as repeated records on the same genotype allows use of the usual mixed model procedures for nonsingular **A** and is computationally simpler and feasible for large populations. Results are shown to be identical to those obtained through use of the more difficult procedures for singular **A**.

INTRODUCTION

Different individuals with identical genotypes can occur in animal populations, as for example through the natural occurrence of identical twins and through the artificial splitting of embryos. Future applications of cloning will result in the production of large groups of animals with identical genotypes. Although members of a clone set have identical genotypes, their phenotypic records will differ unless the trait is perfectly heritable. If genetic evaluations are performed in populations where clones are represented, errors in evaluation will result unless the occurrence of identical genotypes is properly recorded and accommodated. However, existing procedures for accommodating data on identical genotypes are very complicated. Discarding data on identical genotypes results in a loss of information and could introduce bias in genetic evaluations in selected populations. Records associated with each of the identical genotypes should be included.

This paper reviews existing procedures for genetic evaluation when different animals with identical genotypes are represented in the population and presents alternative procedures, under an equivalent model, which lead to the same results but are much easier computationally.

PROCEDURES

Equivalent Models

First we shall consider an additive genetic model with one random factor.

Now consider the model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e} \qquad [1]$$

where **y**, **b**, **a**, and **e** represent vectors of observations on animals, fixed effects, additive genetic (breeding) values of animals and random (environmental) residuals, respectively, and **X** and **Z** are incidence matrices relating observations to fixed effects and to additive genetic values of animals. $E(\mathbf{y}) = \mathbf{Xb}$ and

$$\text{var}\begin{bmatrix} a \\ e \end{bmatrix} = \begin{bmatrix} A\sigma^2_a & 0 \\ 0 & R\sigma^2_e \end{bmatrix}$$

where **A** is the matrix of additive genetic relationships among animals. Then $\text{var}(y) = ZAZ'\sigma^2_a + R\sigma^2_e$.

Genetic evaluation of animals under [1] is usually through solution of Henderson's mixed model equations

$$\begin{bmatrix} X'R^{-1}X & X'R^{-1}Z \\ Z'R^{-1}X & Z'R^{-1}Z + A^{-1}\lambda \end{bmatrix} \begin{bmatrix} \tilde{b} \\ \hat{a} \end{bmatrix} = \begin{bmatrix} X'R^{-1}y \\ Z'R^{-1}y \end{bmatrix} \qquad [2]$$

where $\lambda = \sigma^2_e/\sigma^2_a$.

However, if some of the animals in **a** have identical genotypes, as for example if they were products of the same split embryo, then [2] cannot be used because **A** is singular and A^{-1} does not exist. **A** is singular because rows and columns pertaining to identical genotypes in **A** are identical. Henderson (1986) suggested avoiding A^{-1} by solving

$$\begin{bmatrix} X'R^{-1}X & X'R^{-1}ZG \\ GZ'R^{-1}X & GZ'R^{-1}ZG + G\sigma^2_e \end{bmatrix} \begin{bmatrix} \tilde{b} \\ \tilde{\alpha} \end{bmatrix} = \begin{bmatrix} X'R^{-1}y \\ GZ'R^{-1}y \end{bmatrix} \qquad [3]$$

where $G = A\sigma^2_a$. Additive genetic values are then estimated as $\hat{a} = G\tilde{\alpha}$, and $\hat{a}$ is unique despite a singular **G** and non-uniqueness of $\tilde{\alpha}$. Solution of [3] leads to identical genetic evaluations for identical genotypes. The practical problem is that solution of [3] is much more difficult computationally than solution of [2].

Kennedy and Schaeffer (1989) informally suggested use of an alternative that gives the same $\hat{a}$ as in [3] but allows use of the computationally simpler equations [2]. Under this model a^* is defined such that genetically identical animals are considered as a single genotype and $A^*\sigma^2_a$ represents the variance-covariance matrix among genotypes rather than among animals. Records on different individuals with the same genotype are simply treated as repeated observations on a single genotype. Under this model, A^{*-1} exists and the usual mixed model equations [2] can be used.

The model is

$$y = Xb + Z^*a^* + e \qquad [4]$$

where **y**, **X**, **b**, and **e**, are as defined in [1], a^* is a vector of additive genetic values of genotypes and Z^* is an incidence matrix that connects observations to genotypes. Under this model, $E(y) = Xb$ and $\text{var}(y) = Z^*A^*Z^{*\prime}\sigma^2_a + R\sigma^2_e$, where A^* is the matrix of additive genetic relationships among genotypes.

Models are equivalent if their first and second moments are equal and the following shows the equivalence of [1] and [4]. First partition a' into $(a'_1\ a'_2)$ such that a_1 represents additive genetic values of p animals with unique genotypes and a_2 represents additive genetic values of q animals which have genotypes identical to animals in a_1. We can then express a_2 as a function of a_1 and a matrix P of order q x p such that $a_2 = Pa_1$. Each row of P contains all zero elements except for a one in the column which associates the animal in a_2 with the animal in a_1 of the same genotype. Now

$$\text{var}\begin{bmatrix} a_1 \\ a_2 \end{bmatrix} = \begin{bmatrix} A_{11} & A_{12} \\ A_{21} & A_{22} \end{bmatrix}\sigma^2_a = \begin{bmatrix} A_{11} & A_{11}P' \\ PA_{11} & PA_{11}P' \end{bmatrix}\sigma^2_a$$

where A_{11} is of full rank (p) and A_{12}, A_{21} and A_{22} are linear functions of A_{11}.

Under both [1] and [4], $E(y) = Xb$. Now under [1],

$$y = Xb + Za + e$$

$$= Xb + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix}\begin{bmatrix} a_1 \\ a_2 \end{bmatrix} + e$$

with $\text{var}(y) = ZAZ'\sigma^2_a + R\sigma^2_e$

$$= \begin{bmatrix} Z_1A_{11}Z_1' & Z_1A_{12}Z_2' \\ Z_2A_{21}Z_1' & Z_2A_{22}Z_2' \end{bmatrix}\sigma^2_a + R\sigma^2_e$$

Under [4]

$$y = Xb + Z^*a^* + e$$

but

$$Z^* = \begin{bmatrix} Z_1 \\ Z_2P \end{bmatrix}, \quad a^* = a_1 \text{ and } \text{var}(a^*) = A^*\sigma^2_a = A_{11}\sigma^2_a$$

and therefore

$$\text{var}(y) = \begin{bmatrix} Z_1 \\ Z_2P \end{bmatrix} A_{11} \begin{bmatrix} Z_1 \\ Z_2P \end{bmatrix}' \sigma^2_a + R\sigma^2_e$$

$$- \begin{bmatrix} Z_1 A_{11} Z_1' & Z_1 A_{11} P' Z_2' \\ Z_2 P A_{11} Z_1' & Z_2 P A_{11} P' Z_2' \end{bmatrix} \sigma^2_a + R\sigma^2_e$$

$$- \begin{bmatrix} Z_1 A_{11} Z_1' & Z_1 A_{12} Z_2' \\ Z_2 A_{21} Z_1' & Z_2 A_{22} Z_2' \end{bmatrix} \sigma^2_a + R\sigma^2_e$$

which is the same as under model [1], and the models are equivalent.

The two models can be shown to lead to identical results by first considering the first row of matrices of [3], i.e.

$$X'R^{-1}X\tilde{b} + X'R^{-1}ZG\tilde{a} = X'R^{-1}y. \qquad [5]$$

Since $G\tilde{a} = \hat{a}$ then

$$X'R^{-1}X\tilde{b} + X'R^{-1}Z\hat{a} = X'R^{-1}y.$$

Partitioning **Z** and **a** gives

$$X'R^{-1}X\tilde{b} + X'R^{-1} \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} \hat{a}_1 \\ \hat{a}_2 \end{bmatrix} = X'R^{-1}y$$

and noting from a property of [3] that $Z_2\hat{a}_2 = Z_2P\hat{a}_1$, then [5] becomes

$$X'R^{-1}X\tilde{b} + X'R^{-1}Z^*\hat{a}^* = X'R^{-1}y$$

which is also the first row of [2] under model [4].

Similarly consider the last row of matrices of [3], i.e.

$$GZ'R^{-1}X\tilde{b} + GZ'R^{-1}ZG\tilde{a} + G\tilde{a}\sigma^2_e = GZ'R^{-1}y.$$

Since $\hat{a} = G\tilde{a}$, then

$$GZ'R^{-1}X\tilde{b} + GZ'R^{-1}Z\hat{a} + \hat{a}\sigma^2_e = GZ'R^{-1}y. \qquad [6]$$

Now premultiply both sides of [6] by

$$G^- = \begin{bmatrix} A_{11}^{-1} & 0 \\ 0 & 0 \end{bmatrix} / \sigma^2_a \text{ noting}$$

$$\text{that } G^{-}GZ' = \begin{bmatrix} A_{11}^{-1} & 0 \\ 0 & 0 \end{bmatrix} \begin{bmatrix} A_{11} & A_{12} \\ A_{21} & A_{22} \end{bmatrix} \begin{bmatrix} Z_1' & 0 \\ 0 & Z_2' \end{bmatrix}$$

$$= \begin{bmatrix} Z_1' & A_{11}^{-1}A_{12}Z_2' \\ 0 & 0 \end{bmatrix} = \begin{bmatrix} Z_1' & P'Z_2' \\ 0 & 0 \end{bmatrix}$$

$$= \begin{bmatrix} Z^{*\prime} \\ 0 \end{bmatrix}$$

Then [6] reduces to

$$\begin{bmatrix} Z^{*\prime} \\ 0 \end{bmatrix} R^{-1}\tilde{Xb} + \begin{bmatrix} Z^{*\prime} \\ 0 \end{bmatrix} R^{-1} \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} \hat{a}_1 \\ \hat{a}_2 \end{bmatrix} + \begin{bmatrix} A_{11}^{-1}\lambda & 0 \\ 0 & 0 \end{bmatrix} \begin{bmatrix} \hat{a}_1 \\ \hat{a}_2 \end{bmatrix}$$

$$= \begin{bmatrix} Z^{*\prime} \\ 0 \end{bmatrix} R^{-1}y$$

$$\begin{bmatrix} Z^{*\prime} \\ 0 \end{bmatrix} R^{-1}\tilde{Xb} + \begin{bmatrix} Z^{*\prime} \\ 0 \end{bmatrix} R^{-1} \begin{bmatrix} Z_1 \\ Z_2P \end{bmatrix} \hat{a}_1 + \begin{bmatrix} \lambda A_{11}^{-1} \\ 0 \end{bmatrix} \hat{a}_1$$

$$= \begin{bmatrix} Z^{*\prime} \\ 0 \end{bmatrix} R^{-1}y$$

and

$$\begin{bmatrix} Z^{*\prime} \\ 0 \end{bmatrix} R^{-1}\tilde{Xb} + \begin{bmatrix} Z^{*\prime} \\ 0 \end{bmatrix} R^{-1}Z^{*}\hat{a}^{*} + \begin{bmatrix} \lambda A^{*-1} \\ 0 \end{bmatrix} \hat{a}^{*} = \begin{bmatrix} Z^{*\prime} \\ 0 \end{bmatrix} R^{-1}y$$

which is equivalent to the second row of [2] under model [4], and the two methods lead to identical results.

Numerical Example

The equivalence of the methods in terms of results and the differences between the methods in computational requirements are illustrated by the following example in Figure 1. Animals 4 and 7 are the result of a split embryo and are genetically identical. Animal 5 is a full-sibling to 4 and 7. Also, 6 and 8 are identical genetically and are half-siblings to 4, 5 and 7. The matrix of additive genetic relationships among the animals is

$$\mathbf{A} = \begin{bmatrix} 1 & 0 & 0 & .5 & .5 & .5 & .5 & .5 \\ 0 & 1 & 0 & .5 & .5 & 0 & .5 & 0 \\ 0 & 0 & 1 & 0 & 0 & .5 & 0 & .5 \\ .5 & .5 & 0 & 1 & .5 & .25 & 1 & .25 \\ .5 & .5 & 0 & .5 & 1 & .25 & .5 & .25 \\ .5 & 0 & .5 & .25 & .25 & 1 & .25 & 1 \\ .5 & .5 & 0 & 1 & .5 & .25 & 1 & .25 \\ .5 & 0 & .5 & .25 & .25 & 1 & .25 & 1 \end{bmatrix}.$$

Note that the 4th and 7th rows and columns of **A** are identical as are the 6th and 8th rows and columns, and therefore **A** is singular and $\mathbf{A}^{-1}$ does not exist.

In this example, for simplicity, but with no loss of generality, assume that $\mathbf{R} = \mathbf{I}$ and the only fixed effect is the mean. Also $\lambda = \sigma^2_e/\sigma^2_a = 3$ (i.e. $h^2 = .25$).

Under model [1] based on additive genetic values of animals $\mathbf{a}' = (a_1\ a_2\ a_3\ a_4\ a_5\ a_6\ a_7\ a_8)$

and $\mathbf{Z} = \begin{bmatrix} 0&1&0&0&0&0&0&0 \\ 0&0&1&0&0&0&0&0 \\ 0&0&0&1&0&0&0&0 \\ 0&0&0&0&1&0&0&0 \\ 0&0&0&0&0&1&0&0 \\ 0&0&0&0&0&0&1&0 \\ 0&0&0&0&0&0&0&1 \end{bmatrix}$

Equations [3] (letting $\sigma^2_a = 1$ and $\sigma^2_e = 3$ for convenience) are

$$\begin{bmatrix} 7 & 2.5 & 2.5 & 2.0 & 3.5 & 3.0 & 3.25 & 3.5 & 3.25 \\ 2.5 & 4.25 & .75 & .5 & 3.0 & 2.75 & 2.875 & 3.0 & 2.875 \\ 2.5 & .75 & 4.75 & 0 & 3.25 & 3.0 & .375 & 3.25 & .375 \\ 2.0 & .5 & 0 & 4.5 & .25 & .25 & 3.0 & .25 & 3.0 \\ 3.5 & 3.0 & 3.25 & .25 & 5.625 & 3.375 & 1.875 & 5.625 & 1.875 \\ 3.0 & 2.75 & 3.0 & .25 & 3.375 & 4.875 & 1.75 & 3.375 & 1.75 \\ 3.25 & 2.875 & .375 & 3.0 & 1.875 & 1.75 & 5.4375 & 1.875 & 5.4375 \\ 3.5 & 3.0 & 3.25 & .25 & 5.625 & 3.375 & 1.875 & 5.625 & 1.875 \\ 3.25 & 2.875 & .375 & 3.0 & 1.875 & 1.75 & 5.4375 & 1.875 & 5.4375 \end{bmatrix} \begin{bmatrix} \hat{\mu} \\ \tilde{\alpha}_1 \\ \tilde{\alpha}_2 \\ \tilde{\alpha}_3 \\ \tilde{\alpha}_4 \\ \tilde{\alpha}_5 \\ \tilde{\alpha}_6 \\ \tilde{\alpha}_7 \\ \tilde{\alpha}_8 \end{bmatrix}$$

$$= \begin{bmatrix} 62.0 \\ 21.5 \\ 23.0 \\ 17.5 \\ 31.25 \\ 26.25 \\ 28.0 \\ 31.25 \\ 28.0 \end{bmatrix}.$$

There is no unique solution to these equations but setting $\tilde{\alpha}_7 = \tilde{\alpha}_8 = 0$ leads to a solution

$$(\hat{\mu} \quad \tilde{\alpha}_1 \quad \tilde{\alpha}_2 \quad \tilde{\alpha}_3 \quad \tilde{\alpha}_4 \quad \tilde{\alpha}_5 \quad \tilde{\alpha}_6 \quad \tilde{\alpha}_7 \quad \tilde{\alpha}_8)$$

$$= (8.8870 \quad .0000 \quad .3041 \quad .0462 \quad .0501 \quad -.2571 \quad -.1433 \quad 0 \quad 0).$$

A unique solution for additive genetic values is given by $\hat{\mathbf{a}} = \mathbf{C}\tilde{\boldsymbol{\alpha}}$ and this leads to

$$\hat{\mathbf{a}}' = (\hat{a}_1 \quad \hat{a}_2 \quad \hat{a}_3 \quad \hat{a}_4 \quad \hat{a}_5 \quad \hat{a}_6 \quad \hat{a}_7 \quad \hat{a}_8)$$

$$= (-.1751 \quad .2006 \quad -.0255 \quad .0378 \quad -.1158 \quad -.1720 \quad .0378 \quad -.1720).$$

Note that $\hat{a}_4 = \hat{a}_7$ and $\hat{a}_6 = \hat{a}_8$.

A much simpler solution is obtained under model [4] based on additive genetic values of genotypes and using equations [2], the usual mixed model equations. There are six unique genotypes, a_1 to a_6, and two replicates, a_7 which is identical to a_4 and a_8 which is identical to a_6. Therefore,

$$\mathbf{a}^{*'} = \mathbf{a}_1' = (a_1 \; a_2 \; a_3 \; a_4 \; a_5 \; a_6)$$

and $\mathbf{a}_2' = (a_7 \; a_8)$.

For this example,

$$\mathbf{P} = \begin{bmatrix} 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

and the additive genetic values of animals in $\mathbf{a}_2$ can be expressed in terms of those of animals in $\mathbf{a}_1$ as $\mathbf{a}_2' = \mathbf{a}_1'\mathbf{P}' = (a_4 \; a_6)$. $\mathbf{Z}^*$ can be computed from $\mathbf{Z}$

$$\mathbf{Z}^* = \begin{bmatrix} \mathbf{Z}_1 \\ \mathbf{Z}_2\mathbf{P} \end{bmatrix}$$

where $\mathbf{Z}_1$ is the upper left 5 x 6 and $\mathbf{Z}_2$ is the lower right 2 x 2 submatrix of $\mathbf{Z}$, i.e.

$$Z^* = \begin{bmatrix} 0&1&0&0&0&0 \\ 0&0&1&0&0&0 \\ 0&0&0&1&0&0 \\ 0&0&0&0&1&0 \\ 0&0&0&0&0&1 \\ 0&0&0&1&0&0 \\ 0&0&0&0&0&1 \end{bmatrix} .$$

$A^* = A_{11}$ is the upper left 6 x 6 submatrix of A,

$$A_{11} = \begin{bmatrix} 1&0&0&.5&.5&.5 \\ 0&1&1&.5&.5&0 \\ 0&0&1&0&0&.5 \\ .5&.5&0&1&.5&.25 \\ .5&.5&0&.5&1&.25 \\ .5&0&.5&.25&.25&1 \end{bmatrix} .$$

A_{11} is of full rank and A_{11}^{-1} can be computed easily using Henderson's (1976) simple rules,

$$A_{11}^{-1} = \begin{bmatrix} 2.5&1&.5&-1&-1&-1 \\ 1&2&0&-1&-1&0 \\ .5&0&1.5&0&0&-1 \\ -1&-1&0&2&0&0 \\ -1&-1&0&0&2&0 \\ -1&0&-1&0&0&2 \end{bmatrix} .$$

Application to [2] gives the following equation

$$\begin{bmatrix} 7&0&1&1&2&1&2 \\ 0&7.5&3&1.5&-3&-3&-3 \\ 1&3&7&0&-3&-3&0 \\ 1&1.5&0&5.5&0&0&-3 \\ 2&-3&-3&0&8&0&0 \\ 1&-3&-3&0&0&7&0 \\ 2&-3&0&-3&0&0&8 \end{bmatrix} \begin{bmatrix} \hat{\mu} \\ \hat{a}_1 \\ \hat{a}_2 \\ \hat{a}_3 \\ \hat{a}_4 \\ \hat{a}_5 \\ \hat{a}_6 \end{bmatrix} = \begin{bmatrix} 62 \\ 0 \\ 10 \\ 9 \\ 18 \\ 8 \\ 17 \end{bmatrix}$$

with solutions

$$(\hat{\mu} \quad \hat{a}_1 \quad \hat{a}_2 \quad \hat{a}_3 \quad \hat{a}_4 \quad \hat{a}_5 \quad \hat{a}_6) =$$

(8.8870 -.1751 .2006 -.0255 .0378 -.1158 -.1720).

Solutions for the replicate genotypes $\hat{a}_7$ and $\hat{a}_8$ are obtained from $p\hat{a}_1$, i.e.

$$\begin{bmatrix} \hat{a}_7 \\ \hat{a}_8 \end{bmatrix} = \begin{bmatrix} 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} -.1751 \\ .2006 \\ -.0255 \\ .0378 \\ -.1158 \\ -.1720 \end{bmatrix} = \begin{bmatrix} .0378 \\ -.1720 \end{bmatrix}.$$

Note that all solutions are identical to those obtained under the more complicated computations of [3].

Other Models

Although algebraic and numerical equivalence of the two models and methods was illustrated only for an additive genetic model with one random factor, the equivalence of the models is general and any number of random factors can be accommodated if correctly specified. For example, if some animals had more than one record, permanent environmental effects could be taken into account through correct specification of **R** or through including an additional random effect with its incidence matrix (e.g. **Wp**) in both models [1] and [4]. There would be one element in **p** for each animal in both models. Future production (real producing ability) of the j^{th} clone of the i^{th} genotype would be predicted as $\hat{a}_i + \hat{p}_{ij}$ under [4]. Also, if nonadditive genetic effects such as dominance were important, both models could be expanded to incorporate them leading to identical results. Under [1] dominance effects would be expressed at the animal level, with a singular dominance genetic variance-covariance matrix, and under [4] dominance effects would be expressed at the genotypic level, with a nonsingular dominance genetic variance-covariance matrix. The procedures are also applicable to multiple trait evaluation.

Henderson (1986) has also proposed use of [3] for variance component estimation by minimum variance quadratic unbiased estimation (MIVQUE) and restricted maximum likelihood (REML). Again, a computationally simpler alternative is to model at the genotype level and use equation [2]. Estimates of variances will be identical.

Practical Implications

Split zygotes and other forms of clones can be accommodated in genetic evaluation under animal models by simple modifications of **Z** and **A** which then allow use of the usual mixed model equations incorporating $\mathbf{A}^{-1}$. Therefore, the presence of genetic clones in a population does not present a major problem for genetic evaluation as would be the case if equations for singular **G** had to be used. However, if identical genotypes are present, recording and registration programs must identify them as such. Otherwise identical genotypes will be treated as full-sibs under an animal model, or possibly as unrelated under some sire evaluation models. Over and above the loss of accuracy in genetic evaluation and possible bias if selection has occurred, this will lead to different genetic

evaluations for genetically identical animals which may result in loss of credibility for the genetic evaluation system.

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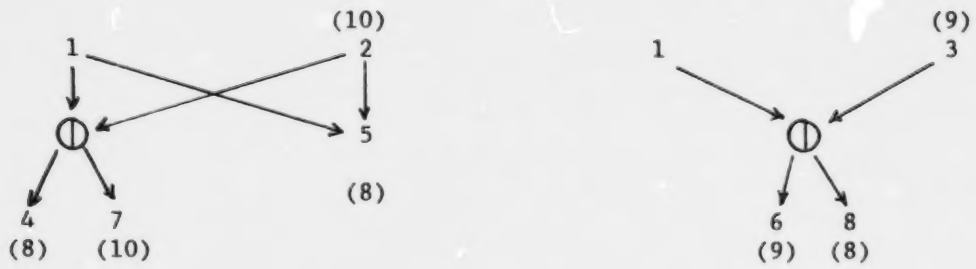
Figure 1. Pedigrees (animal numbers) with phenotypic records (in parentheses) for the numerical example. (ⓘ represents a split embryo).



COMPARISON OF THEORETICAL AND SIMULATED EQUILIBRIUM GENETIC RESPONSE RATES WITH PROGENY TESTING IN DAIRY CATTLE

K. Meyer and C. Smith
Centre for Genetic Improvement of Livestock, Department of Animal & Poultry Science, University of Guelph

SUMMARY

The published theoretical estimates of equilibrium genetic change possible by progeny testing in dairy cattle are too high. Much of the overestimation is because no account has been taken of the temporary reduction in genetic variation (Bulmer, 1971) due to selection of parents and ancestors. A particularly efficient progeny testing scheme was modelled theoretically and simulated on computer using a continuous infinitesimal additive model. To make the simulation feasible an elite breeding herd of females was used, and progeny testing was done outside the herd. The proportional reduction in genetic variance due to previous selection was 0.25, 0.27 and 0.29 for original heritabilities of 0.05, 0.25 and 0.50, with the theoretical and simulated results being very similar. The theoretical and simulated proportional losses in equilibrium selection responses were 0.22 and 0.26, 0.21 and 0.22 and 0.21 and 0.25, respectively, for the three levels of heritability. Thus, most of the loss in response in the simulation results was due to the reduction in genetic variance due to selection.

INTRODUCTION

Robertson and Rendel (1950) estimated the theoretical rate of genetic change possible in dairy cattle by artificial insemination and progeny testing, and their method has been widely used in the literature. Bulmer (1971) showed that, with an infinitesimal additive genetic model, the genetic variation is temporarily reduced by selection of parents and ancestors, but regenerates quickly when the selection is relaxed. Bulmer (1971) called this "joint disequilibrium" while Falconer (1981) used "gamete phase disequilibrium". For simplicity it will be termed here the reduction in genetic variance due to selection.

The reduction in genetic variance in a selected stock results in a reduction in the measured heritability in the stock (Fimland, 1979) and in the genetic response rate. The reduction in response rate can be appreciable, as shown by Bulmer (1971) and Falconer (1981) for mass selection of a highly heritable trait. Several computer simulations of small nucleus MOET (multiple ovulation and embryo transfer) schemes in dairy cattle have found genetic responses appreciably lower than the theoretical rates (Juga and Maki-Tanila, 1987; J. Jansen and W. Schlote, and M. Toro, personal communications). The objective here was to simulate a large progeny testing scheme and estimate the equivalent reduction in theoretical response. To reduce the simulation to a suitable size a progeny testing scheme with a closed elite nucleus herd of breeding females, and testing young bulls outside the nucleus herd was used. This scheme theoretically gives higher rates of genetic response than the conventional schemes with a large field population of breeding females (Woolliams and Smith, 1988).

PROCEDURES

The progeny testing scheme tested 150 young bulls per year outside the nucleus breeding unit, that is in the commercial or base population. Five bulls were selected on progeny test results per year, and used for one year. They were used to breed both the young bulls for testing and the young females as replacements in the nucleus unit, so bulls to breed bulls (BB) were identical to bulls to breed cows (BC). The elite breeding nucleus consisted of 2,525 calves plus 8,000 females mated, made up of 2,273, 2,091, 1,568, 1,182 and 886 at 1, 2, 3, 4 and 5 years of age, respectively. These correspond to a 92% calf survival rate, a random 75% cow survival rate from one year to the next, and a 90% chance that a female produces a live calf. Cows were culled after 4 lactations. Cows with 1, 2 or 3 records (as available) were indexed and the best (375/3,636) selected to breed young bulls. The index of these cows to breed bulls (CB) included their own records and the progeny test BLUP (Best Linear Unbiased Prediction) of their sire. Replacement females came at

random from all matings in the nucleus herd (CC). The generation intervals were 6 years for bulls (BB, BC), 4.812 for CB and 3.151 for CC (see Appendix 2). To cover a range of traits, heritabilities of 0.05, 0.25 (milk yield) and 0.50 were used, with corresponding repeatabilities of 0.3, 0.5 and 0.75. The number of effective daughters in the progeny test were 316, 60 and 28, respectively, for the three heritabilities, corresponding to an accuracy of 0.894 for the progeny test.

Bull proofs were obtained by BLUP with a sire model, accounting for relationships to sires and maternal grandsires. The linear model included annual genetic groups as the only fixed effects, with an additional group effect used for proven bulls used in years 0 to 3. The BLUP breeding values were 2 x (sire solution + group solution). Twenty replicates were run and the results were averaged over replicates.

For the starting conditions it was assumed that a female population, with the age structure described, was already in existence. The bulls in years 0-3 were from the same base, progeny tested on the specified number of daughters, and the best 5/150 were used for breeding. Interest was in the equilibrium genetic variances and rates of response rather than in the short term means.

The theoretical genetic variance with selection over the years, was calculated deterministically by computer, using the sequential methods outlined by Bulmer (1971). The details are in Appendix 1. The equilibrium genetic variance with a progeny test can also be derived algebraically (Gomez-Raya, 1988). The theoretical rates of response with the original genetic parameters and with the adjusted genetic parameters were then calculated as in Robertson and Rendel (1950) and compared with the simulated results.

RESULTS AND DISCUSSION

With cow records and progeny test results available in the first years, the theoretical genetic variances fell sharply in the first generation and reached an equilibrium quickly, as shown in Figure 1 for an original genetic variance (heritability) of 0.25. The proportional reduction in genetic variance was 0.25, 0.27 and 0.29 for the three levels of heritability (0.05, 0.25, 0.50). The results from the computer simulation were very similar, although slightly less for males and more for females. The corresponding heritabilities fell proportionally by 0.24, 0.22 and 0.17.

The equilibrium parameter estimates were then used to recalculate the accuracy of sire and dam selection. For sire selection the accuracies fell from 0.894 to 0.854, 0.860 and 0.865 for the three levels of heritability. The fall in accuracy of dam selection was greater because proportionally more information comes from the sire's progeny test, and the sires are highly selected so there is less genetic variation among them. The dam accuracies fell from 0.495, 0.640 and 0.770 to 0.300, 0.521 and 0.697, respectively, for the three levels of heritability.

The genetic trends were then re-estimated, using the revised parameters, by the conventional method and an example is given in Appendix 2. With the small population selection intensity for bulls (2.158 vs. 2.225), the estimated response rate fell proportionately by 0.22, 0.21 and 0.21 for the three levels of heritability.

In the simulation, the behaviour of both the genetic variance and the genetic trend was very stable, as judged by the standard deviation of replicate results. For example, for the 0.25 heritability, the mean response after 20 years was about two (original) standard deviation units, and the standard deviation of this result among replicates was 0.08. The stability is partly due to the large number of females mated (8000) per replicate. There was more variation in the genetic merit of the five bulls per year selected (on estimated breeding value) but this effect was moderated by having 25 bulls per generation (5 years).

The simulated annual genetic responses also reached equilibrium values quickly as illustrated in Figure 1, but were somewhat lower than the theoretical equilibrium responses with the revised parameters. The proportional losses in response were 0.26, 0.22 and 0.25 for the three levels of heritability. Put the other way around, the proportional overestimations by the conventional methods were 0.35, 0.28 and

0.33, respectively.

The motivation for this study was the results from simulation of MOET nucleus schemes in dairy cattle. In several simulation studies the genetic responses estimated were appreciably less than the theoretical estimates derived by Nicholas and Smith, 1983. Losses in expected response in simulations of MOET schemes were 35-55 percent (Juga and Maki-Tanila, 1987) and 26-33 percent (J. Jansen and W. Schlote, personal communication) for adult MOET schemes, and 14-59 percent (M. Toro, personal communication) for juvenile MOET schemes. The larger losses were for schemes with few (1-4) sires or with small herd size (256-512 embryo transfers) or both. With less accurate and less intense selection than for a progeny test scheme, the reduction in genetic variance due to selection will be less in MOET schemes. But with more reliance on collateral relatives (full sibs and half sibs) there is a higher correlation of index values of family members, and this reduces the effective selection differentials in small populations (Hill, 1977). Woolliams (1988) has estimated this would cause a proportional loss in response of about 0.15 in small MOET schemes.

There seemed to be no equivalent simulation results with progeny testing, presumably because of the size of the population of females involved in national progeny testing systems. To reduce the simulation problem to a reasonable size, a system using an elite breeding nucleus of females, all bred to the best progeny tested sires (Woolliams and Smith, 1988), and a sire model (rather than an animal model) were used. By progeny testing young bulls outside the nucleus, the size of the nucleus is smaller. The system is also simpler to specify theoretically. The effect of reduction in genetic variance due to selection on the selection response does not seem to have been studied theoretically in dairy cattle improvement, though Fimland (1979) evaluated the reduction in genetic variances expected and their effects on the accuracy of bull proofs.

The sire model required less time and space in computing, than an animal model, but caused some other problems. The same ratios for $(V_R/(0.25V_A))$ were used in the BLUP equations over the generations, rather than using the adjusted higher current values (which would have to be estimated). This will tend to regress the estimates of breeding value less than they should be. However, with the structure of the testing system, all the bulls have the same information so their ranking should not be affected. Relationships between sire and grandsire was included but not all relationships were used as is done with an animal model. With the animal model the accuracy of evaluation is increased but the correlation of index values among relatives is also increased, reducing the effective selection differentials (Hill, 1977). Inbreeding was ignored but should be small with 25 sires per generation of 5 years. The theoretical rate of inbreeding is from 0.001 to 0.002 per year, giving a maximum of 0.05 percent after 25 years. The reduction in genetic variance due to this inbreeding and the inbreeding depression on performance will both further reduce the genetic response rates in practice.

The models used here are simplistic, assuming many loci with small additive effects. For some traits there may be genes with large effect, or appreciable non-additive genetic variance. In practice there are many other factors which affect response rates, such as selection for several traits rather than only one, inaccuracies of bull dam selection, long generation intervals and less selection practiced than is possible (Van Vleck, 1988). In the past, parameters have been estimated in populations under selection, so the true genetic variance may be underestimated, and the reduction in genetic variance due to selection may already be partly discounted. With an animal model used in analysis, the parameters in the base population are estimated, so the reduction in genetic variance due to selection will have to be taken into account in the future.

Van Vleck (1986, 1988) has reviewed the rates of genetic change being achieved by successful progeny testing schemes for milk yield. The rates have improved appreciably over the last decade and are now quite high. They range from 0.05 to 0.10 standard deviation units per year. The latter equals the equilibrium simulated response (heritability 0.25) with the efficient progeny testing schemes used here. On re-appraisal then, current progeny testing

schemes in North America may be more efficient than considered previously.

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APPENDIX 1.

Changes in genetic variation with continuous selection

Original: Phenotypic variance $V = 1$
Genetic (additive) variance V_A = Heritability
Residual variance V_R
Common variance V_C, Repeatability = $V_A + V_C$
B = Bull, C = Cow
x and i are the normal deviate and selection intensity corresponding to the proportion selected
$k = i(i-x)$

General formula

Variance in N after selection on M = $VN - k(CovMN)^2/VM$

Genetic variance in the next generation

$$V_A = 0.25\ V_A(\text{Bulls}) + 0.25\ V_A(\text{Cows}) + 0.5\ V_A$$

Variance of means

Variance of cow mean $\bar{Y} = V_{\bar{Y}} = (V_R + (m-1)V_C + mV_{AC})/m$
(m records)
Variance of bull progeny mean $\bar{X} = V_{\bar{X}} = (V_R + 0.75\ V_A + nV_{AB})/n$
(n progeny)

Yearly genetic variances

Bulls born in year t:

$$V_{AB_t} = 0.25\ V_{ABS_{t-5}} + 0.25\ V_{ACS_{t-4}} + 0.5\ V_A$$

Cows born in year t

$$V_{AC_k} = 0.25\ V_{ABS_{t-5}} + 0.25\ V_{AC_{t-2}} + 0.5\ V_A$$

Selected bulls

$$V_{ABS_t} = V_{AB_t} - (0.5\ V_{AB_t})^2 k_B / V_{\bar{X}_t}$$

Selected cows

$$V_{ACS_t} = V_{AC_t} - (V_{AC_t})^2 k_C / V_{\bar{Y}_t}$$

APPENDIX 2.

Equilibrium theoretical annual genetic response (Robertson and Rendel, 1950))

$$\Delta G = \frac{(i_{BB}r_{BB} + i_{BC}r_{BC} + i_{CB}r_{CB} + i_{CC}r_{CC})\sqrt{V_A}}{L_{BB} + L_{BC} + L_{CB} + L_{CC}} \sigma$$

i = selection intensity

r = accuracy of selection

L = age of parents at the birth of their progeny

Selection; Males 5/150, $i_{5/150}$ = 2.225 ($i_{5/150}$ = 2.185)
Females 375/3636, i = 1.741

Heritability 0.25, Repeatability 0.5
Accuracy of progeny test on 60 daughters; 0.8944
Accuracy (weighted) of dam index 1, 2, 3 records + 60 half sibs; 0.640

$L_{BB} = L_{BC} = 6$

L_{CB} = (1568x4 + 1182x5 + 836x6)/3636 = 4.812

L_{CC} = (2091x2 + 1568x3 + 1182x4 + 883x5)/3636 = 3.151

$$\Delta G = \frac{(2.225(0.8944) + 2.225(0.8944) + 1.741(0.640) + 0)0.5}{6 + 6 + 4.812 + 3.151} \sigma$$

= 0.128 s.d. units

Equilibrium theoretical annual genetic response, adjusted for reduced genetic variance

(r_{BB} = 0.8598 V_{AB} = 0.1772 i_{BB} = 2.185

r_{CB} = 0.5209 V_{AC} = 0.1878)

Adjusted ΔG = 0.098 s.d. units

Table 1. Theoretical and simulated parameters and responses for the progeny testing system modelled.

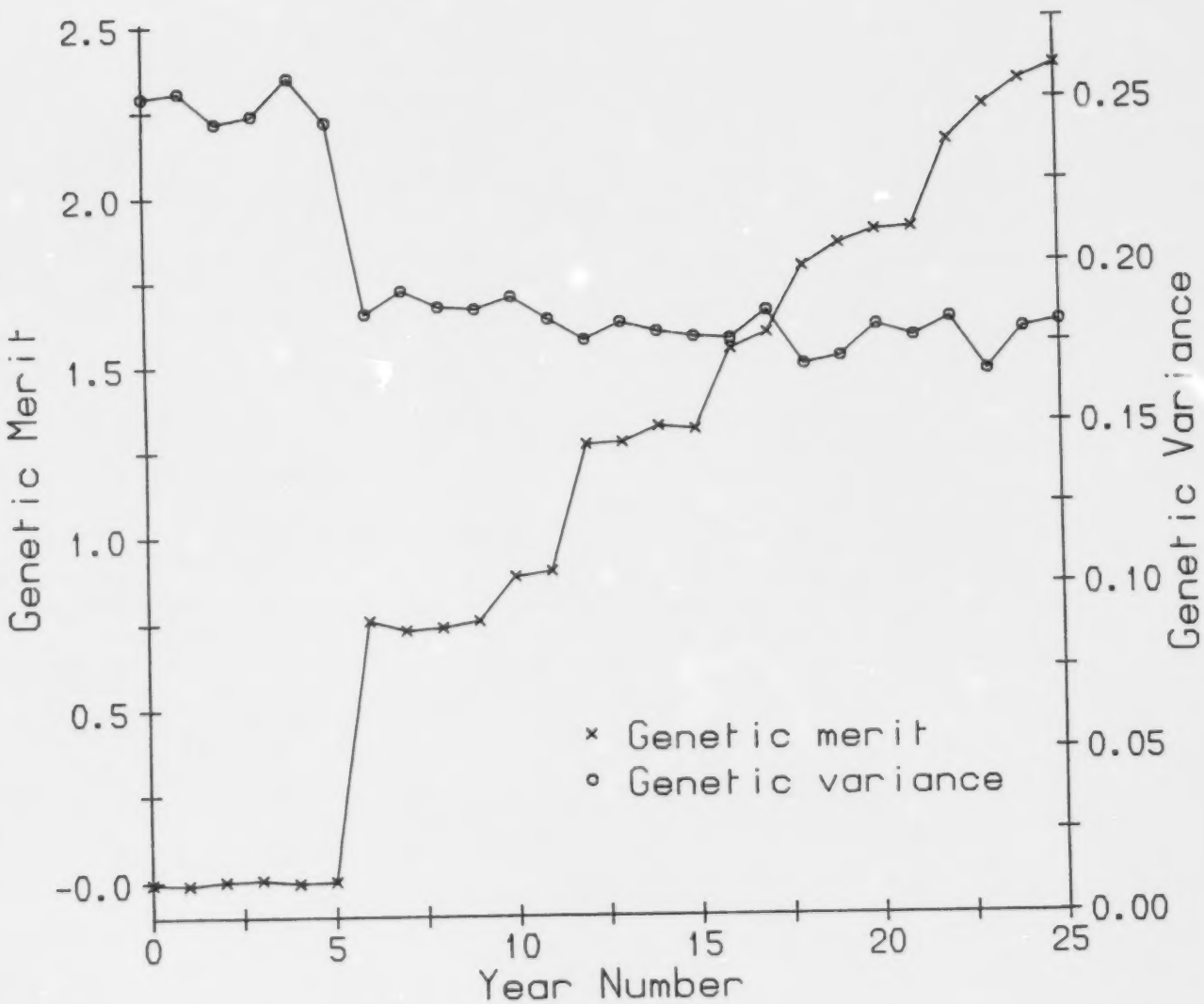
Heritability (Repeatability)		Genetic variance M	Genetic variance F	Heritability M	Heritability F	Equilibrium annual genetic response i_{∞}	Equilibrium annual genetic response $i_{5/150}$	Proportional loss in response
0.05	1	0.05	0.05	0.05	0.05	0.0542	0.0534	----
(0.30)	2	0.0372	0.0376	0.0377	0.0381	0.0423	0.0416	0.22
	3	0.0366	0.0379	0.0371	0.0384	-------	0.0396	0.26
0.25	1	0.25	0.25	0.25	0.25	0.1276	0.1258	----
(0.50)	2	0.1772	0.1878	0.1911	0.2003	0.1004	0.0989	0.21
	3	0.1750	0.1912	0.1892	0.2031	-------	0.0977	0.22
0.5	1	0.5	0.5	0.5	0.5	0.1885	0.1860	----
(0.75)	2	0.3357	0.3745	0.4017	0.4282	0.1474	0.1455	0.21
	3	0.3298	0.3784	0.3974	0.4308	-------	0.1402	0.25

1 Original parameters

2 Theoretical equilibrium parameters

3 Simulation equilibrium parameters

Figure 1. Trends in the genetic variance and in genetic merit (in original standard deviation units) in males, by year of birth. The points are the mean of 20 replicates. (Heritability, 0.25)



PARAMETER ESTIMATES FOR FEED INTAKE AND PRODUCTION IN FIRST LACTATION USING MILK RECORDING DATA

R.K. Moore [1,2], B.W. Kennedy[2], L.R. Schaeffer[2] and J.E. Moxley[1]
[1]Dairy Herd Analysis Service, Macdonald College of McGill University, [2]Centre for Genetic Improvement of Livestock, Department of Animal and Poultry Science, University of Guelph

SUMMARY

Individual cow test-day records collected between December, 1979 and June, 1986 were used to generate part lactation feed intake and 4% fat corrected milk (FCM) measures for 9,361 Ayrshire and 93,336 Holstein cows in first lactation. Grain energy and total energy intake and FCM measures were taken for the first 70 and 90 days of lactation and from days 70 to 305 and days 90 to 305. Separate analyses by breed were carried out according to a multiple trait mixed model. Sixty-six Ayrshire and 416 Holstein sires were treated as random in the analyses. Heritability estimates for both grain and total energy intake in early lactation were from .04 to .06 for Ayrshires and .10 to .13 in Holsteins. Heritabilities were between .12 and .18 for the corresponding measures in both breeds for the late lactation period. Genetic correlations between energy intake and FCM within a time period were .79 and higher for the early period and .93 and higher for the later lactation period. The genetic correlations between FCM in the early period and both feed intake and production in the later period were higher than those between feed intake in early lactation with the later period intake and production measures.

INTRODUCTION

Feed intake represents a major part of the cost of milk production. Present knowledge of its genetic parameters comes largely from relatively small experimental herds and not from larger commercial populations. Relatively large numbers of cows are needed to measure genetic differences, and milk records from commercial herds can provide this. Further, if any use is to be made of the information in practical selection, the measure must be relatively easy to use so that enough cows can be measured to practice selection effectively. Thus, the potential value of field data on feed intake needs to be assessed.

The objective of the study was to use milk recording data to estimate genetic parameters of feed intake and its relationship to production using a multiple trait model. The present study examines these relationships both within and across the early and later lactation periods.

PROCEDURES

Data Collection

Approximately 7.5 million individual test-day records for a large subpopulation of Quebec DHAS-PATLQ herds were accumulated from December, 1979 to June, 1986. Herds were retained on the basis of having a minimum 25 percent of the cows of the herd identified as to sire and with at least 75 percent of all input feeds being reported with complete chemical analysis. This included the net energy of lactation (NEL) and protein percentage of each feed. Monthly records from 1,922 Holstein and 201 Ayrshire herds were selected.

Test day records for individual cows contained the amount of grain and protein supplement fed in addition to milk produced and its fat and protein percentages. Forages were fed as part of the base ration for up to four different feeding groups in the herd.

Measures of feed intake retained initially were the energy intake (Mcal), protein intake (kg) and dry matter intake (kg) for grain as well as the total of the grain and base ration intakes for these same three measures. Cumulative lactation measures for these traits and 4% fat corrected milk (FCM) production were calculated at 70, 90 and 305 days of lactation. Intakes from the base ration were calculated and accumulated in the same manner as used by DHAS-PATLQ to compute grain and protein supplement recommendations and to calculate feeding indexes.

Records on a total of 156,874 Holstein and 16,198 Ayrshire first lactation cows were

generated from the available test-day data prior to data editing.

Data Editing

The data set was restricted so that only records on daughters of sires that had been sampled through the Centre d'Insemination Artificielle du Quebec (CIAQ) were retained. Also, a restriction was imposed that sires had a minimum of 10 records in 10 herds.

Records on cows of less than 70 days duration or on cows that changed herds were discarded as were 337 records for cows identified as initiating their first lactation after 40 months of age. Finally, records for cows that completed the early period but not the later due only to truncation of the data set were not retained. The edited data contained records on 93,336 Holstein and 9,631 Ayrshire first lactation cows.

Data Analysis

Correlations between the measures of energy, protein and dry matter for grain and total intake either were or closely approached unity. On this basis, only the measures of grain energy intake and total energy intake were retained for further analysis.

Cows that had 70 or 90 day feed intake and production records did not necessarily have 305 day records. A maximum likelihood related multi-trait procedure, which allows for sequential culling of records, was used to obtain variance and covariance estimates involving early and later lactation measures. The model for each trait was:

$$Y_{ijklm} = \mu + hys_i + a_j + g_k + S_{kl} + e_{ijklm}$$

where Y_{ijklm} is the ijklmth observation for the trait, μ is the population mean, hys_i is the fixed effect of the ith herd-year-season of calving (HYS subclasses were March 1 to August 31 and September 1 to February 28 with an average of approximately 4.5 records per HYS, which was greater than if HYS of birth had been used), a_j is the fixed effect of the jth age of cow at calving (20 monthly subclasses from $\leq$ 21 months to 40 months of age), g_k is the fixed effect of the kth sire group (random sires were allocated to one of six groups based on year of first daughter calving and all fixed sires were assigned to a seventh group), S_{kl} is the effect of the lth sire within the kth group (proven sires were treated as fixed while sampling sires were considered as random) and e_{ijklm} is the random residual associated with the ijklmth cow.

Four different four-trait analyses were run. Either grain energy intake or total intake measured in early lactation (70 day or 90 day) was included with FCM in this same period as well as the energy intake on the same basis and FCM for the corresponding later lactation period (70-305 day or 90-305 day).

Paternal half-sib heritability estimates and genetic and phenotypic correlations were computed from the appropriate estimates of sire and residual variances and covariances.

RESULTS AND DISCUSSION

Heritability

Heritabilities of grain and total energy intake and FCM for the different early and late lactation periods for the two breeds are in Table 1.

Heritability estimates for grain energy and total energy intake were essentially the same for the early lactation period, and ranged from .04 to .06 for Ayrshires and .10 to .13 for Holsteins. Heritabilities for these measures were higher for Ayrshires in the late lactation period (.13 to .18), but only slightly greater for Holsteins (.12 to .16).

Heritability estimates for FCM were higher for the measures of early lactation, with larger values estimated for the Holstein population for both of the periods. Heritability estimates for FCM for the 70-305 day period were lower than for the 90-305 day period (.08 vs .13 and .13 vs .21 for Ayrshires and Holsteins, respectively). This appeared to be due to a larger residual variance for the longer time period rather than differences in the additive genetic variance. This would suggest a high degree of variability for FCM in the 70-

90 day period of lactation.

Phenotypic Correlations

Estimates of the phenotypic correlations between grain energy intake and FCM for the two definitions of early and late lactation are in Tables 2 and 3. The values for total energy intake and FCM are presented in Tables 4 and 5. With one exception, the correlations ranged between .50 and .72 in Holsteins. Correlations for the Ayrshire breed were similar, the exceptions being that correlations between the early measures of both intake and FCM with the later lactation measures of energy intake tended to be lower (.30 to .46).

In general, there was little difference between the correlations for early energy intake or early FCM with the two later measures in the present study. Only small differences were observed for correlations with grain energy versus total energy within the same breed and time period.

The phenotypic correlations between the 70-305 day grain energy intake and 70-305 day FCM were considerably lower than for the same measures from 90-305 days (.16 vs .47 in Ayrshires and .36 vs .67 in Holsteins). As mentioned earlier, considerable variation in the estimates for the later part of lactation occurs whether the period from 70 to 90 days is included or not. This is the period when dairymen are making large adjustments in the allocation of grain to individual cows based on production and body condition. The relationship of being fed according to production appears higher for the later period of lactation when this transition period is not included.

The present study based on milk recording data does not clarify understanding of the interplay between cause and effect of intake and production. However, the phenotypic correlations between intake and yield from these data support the observation that high milk yield is associated with generous grain feeding and high total nutrient intake.

Genetic Correlations

Tables 6 through 9 contain estimates of the genetic correlations between the different measures of feed intake and FCM. All correlations between energy intake and FCM within either the early or later lactation period were very high, especially for the later period where the estimates ranged between .93 and .98. Correlations for Ayrshires between intake and FCM using either the 70 day or 90 day period ranged between .84 and .88 for both grain and total energy. Total energy intake correlations with FCM in Holsteins were slightly lower in these early periods, .79 and .80, than corresponding estimates with grain energy intake of .93.

FCM for both the 70 day and 90 day measures of early lactation was highly correlated with both intake and production in later lactation for both breeds, the values ranged from .72 to .91. Genetic correlations between both grain and total energy intakes in early lactation with the later measures were lower than with the early production. They were similar in magnitude to the phenotypic correlations. In all other instances, the genetic correlations were higher than the corresponding phenotypic correlations.

CONCLUSIONS

Differences in genetic parameters for feed intake and production estimated for the Ayrshire and Holstein breeds were small. The genetic correlations suggest that daughters of high production sires are also the largest consumers of energy. If the practice of feeding according to production is responsible for the high genetic correlations observed between feed intake and yield, it would be preferable to use the 70 day feed intake measure rather than the 90 day measure as the phenotypic correlation with FCM was somewhat lower. However, the difference in genetic correlation between 70 and 90 day intake and production was only very slightly lower for the earlier measures. The results of this study for early lactation may reflect the fact that decisions as to how cows should be fed based on production are made very early in the lactation. The results of the present study suggest that any adjustment of production records for feed intake measured through milk recording would lead to a partial correction of milk production for milk production.

Finally, the lower heritability of feed intake than yield and the high genetic correlation with yield would suggest little benefit from including feed intake as presently measured through milk recording, as a selection criterion. Feeding systems which allow for the computerized collection of individual animal intakes of both forage and grain may in the future, however, provide data that could possibly be useful for selection.

ACKNOWLEDGEMENTS

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Table 1. Heritabilities of energy intake and FCM in early and late lactation

Trait	Breed	
	Ayrshire	Holstein
70 day grain energy	.05	.10
90 day grain energy	.06	.13
70 day total energy	.04	.10
90 day total energy	.04	.12
70 day FCM[1]	.17	.26
90 day FCM[1]	.17	.28
70-305 day grain energy	.18	.12
90-305 day grain energy	.18	.14
70-305 day total energy	.12	.14
90-305 day total energy	.13	.16
70-305 day FCM[1]	.08	.13
90-305 day FCM[1]	.13	.21

[1] Average of the two separate estimates when included with grain or total energy.

Table 2. Phenotypic correlations between 70 day and 70-305 day grain energy intake and FCM in Ayrshires and Holsteins[1]

	Trait	1	2	3	4
1.	70 day grain energy	-	.55	.45	.55
2.	70 day FCM	.53	-	.46	.55
3.	70-305 day grain energy	.64	.65	-	.16
4.	70-305 day FCM	.55	.55	.36	-

[1] Ayrshire correlations are above the diagonal and Holstein correlations are below the diagonal.

Table 3. Phenotypic correlations between 90 day and 90-305 day grain energy intake and FCM in Ayrshires and Holsteins[1]

	Trait	1	2	3	4
1.	90 day grain energy	-	.62	.41	.66
2.	90 day FCM	.60	-	.42	.66
3.	90-305 day grain energy	.62	.62	-	.47
4.	90-305 day FCM	.67	.68	.67	-

[1] Ayrshire correlations are above the diagonal and Holstein correlations are below the diagonal.

Table 4. Phenotypic correlations between 70 day and 70-305 day total energy intake and FCM in Ayrshires and Holsteins[1]

	Trait	1	2	3	4
1.	70 day total energy	-	.49	.34	.58
2.	70 day FCM	.55	-	.39	.67
3.	70-305 day total energy	.53	.61	-	.32
4.	70-305 day FCM	.60	.69	.54	-

[1] Ayrshire correlations are above the diagonal and Holstein correlations are below the diagonal.

Table 5. Phenotypic correlations between 90 day and 90-305 day total energy intake and FCM in Ayrshires and Holsteins[1]

	Trait	1	2	3	4
1.	90 day total energy	-	.63	.30	.61
2.	90 day FCM	.62	-	.34	.69
3.	90-305 day total energy	.50	.57	-	.37
4.	90-305 day FCM	.63	.72	.68	-

[1] Ayrshire correlations are above the diagonal and Holstein correlations are below the diagonal.

Table 6. Genetic correlations between 70 day and 70-305 day grain energy intake and FCM in Ayrshires and Holsteins[1]

	Trait	1	2	3	4
1.	70 day grain energy		.84	.40	.49
2.	70 day FCM	.93	-	.77	.83
3.	70-305 day grain energy	.54	.74	-	.94
4.	70-305 day FCM	.56	.77	.98	-

[1] Ayrshire correlations are above the diagonal and Holstein correlations are below the diagonal.

Table 7. Genetic correlations between 90 day and 90-305 day grain energy intake and FCM in Ayrshires and Holsteins[1]

	Trait	1	2	3	4
1.	90 day grain energy	-	.88	.56	.61
2.	90 day FCM	.93	-	.83	.87
3.	90-305 day grain energy	.63	.78	-	.97
4.	90-305 day FCM	.60	.78	.98	-

[1] Ayrshire correlations are above the diagonal and Holstein correlations are below the diagonal.

Table 8. Genetic correlations between 70 day and 70-305 day total energy intake and FCM in Ayrshires and Holsteins[1]

	Trait	1	2	3	4
1.	70 day total energy	-	.87	.69	.67
2.	70 day FCM	.79	-	.88	.88
3.	70-305 day total energy	.58	.72	-	.97
4.	70-305 day FCM	.47	.75	.98	-

[1] Ayrshire correlations are above the diagonal and Holstein correlations are below the diagonal.

Table 9. Genetic correlations between 90 day and 90-305 day total energy intake and FCM in Ayrshires and Holsteins[1]

	Trait	1	2	3	4
1.	90 day total energy	-	.87	.73	.75
2.	90 day FCM	.80	-	.88	.91
3.	90-305 day total energy	.68	.77	-	.97
4.	90-305 day FCM	.54	.79	.93	-

[1] Ayrshire correlations are above the diagonal and Holstein correlations are below the diagonal.

RELATIONSHIPS BETWEEN REPRODUCTION TRAITS, AGE AND BODY WEIGHT AT CALVING AND DAYS DRY IN FIRST LACTATION AYRSHIRES AND HOLSTEINS

R.K. Moore[1,2], B.W. Kennedy[2], L.R. Schaeffer[2] and J.E. Moxley[1]
[1]Dairy Herd Analysis Service, Macdonald College of McGill University and [2]Centre for Genetic Improvement of Livestock, Department of Animal and Poultry Science, University of Guelph

SUMMARY

Individual cow test-day records collected between December, 1979 and June, 1986 were used to calculate measures of reproductive performance, age and weight at calving and days dry for 7,824 Ayrshire and 79,755 Holstein cows in first lactation. Separate analyses by breed were carried out according to a multiple trait mixed model. Sixty-two Ayrshire and 369 Holstein sires were treated as random in the analyses. Ayrshires were on average older and lighter at calving than Holsteins, but the breeds differed little in reproduction measures and days dry. Heritabilities of fertility traits, days to first breeding, days open and services per conception, were all less than .015 in the multi-trait analyses. With the exception of body weight, heritability estimates for the other traits were less than .05. Phenotypic correlations between traits were almost identical for the two breeds and genetic correlations tended to be similar. Exceptions included days to first breeding and services per conception, but heritabilities of these traits were close to zero (<.008) in Ayrshires. Fertility traits were positively correlated genetically. Genetic correlations between days open and both age and body weight at calving were small. The genetic correlation between age and weight at calving was -.90 and -.68 in Ayrshires and Holsteins, respectively. Days dry was moderately positively genetically correlated with all traits except body weight.

INTRODUCTION

Reproductive efficiency has an effect on profit derived from milk and must be considered if the goal is to improve total performance of the cow and to maximize economic performance of a herd.

The objective of this study was to estimate genetic parameters of measures of fertility and their relationships to each other and to weight and age at calving and length of dry period in first lactation Ayrshire and Holstein cows using a multiple trait mixed model.

PROCEDURES

Data Collection

Approximately 7.5 million individual test-day records for a large sub-population of Quebec Dairy Herd Analysis Service - Programme d'Analyse des Troupeaux Laitiers du Quebec (DHAS-PATLQ) herds were accumulated from December, 1979 to June, 1986.

Test day records for individual cows contained the number of breedings and the last breeding date reported by the dairyman since the previous test. Body weight, in the majority of cases an estimate based on a heart girth measurement taken after calving, is reported on the first test after calving for first lactation cows. Dry dates and subsequent calving dates are reported on the first test following their occurrence.

Lactation measures of reproductive performance were days to first breeding (DFB), days open (DO), services per conception (SC), and gestation length (GL). Other measures were age at calving (AGE), body weight (BW) and days dry (DD).

Data Editing

Only first lactation records were used. Editing was done to restrict the data to sires that had been sampled through the Centre d'Insemination Artificielle du Quebec (CIAQ) and for other conditions. First lactations that were ongoing when data collection stopped were not included if the calving date was after January 1, 1985. This was done so that all cows would have at least one and a half years to express the traits under study. Days open and services per conception were calculated for culled animals if they were more than 150 days in

gestation when they left the herd. Missing first breeding dates arise when more than one date is reported for a test interval, as the practice at DHAS has been to store only the most recent date. Records were deleted for cows that were bred but whose first breeding date was missing. The edited data contained records on 7,824 Ayrshire and 79,755 Holstein cows.

Data Analysis

For estimation of variance components, effects of sires on records of sampling daughters were considered random while effects of proven sires on their daughter records were treated as fixed. Sires which had an evaluation prior to December, 1979 were considered as proven. There were 24 Ayrshire and 79 Holstein sires treated as fixed with 62 Ayrshire and 369 Holstein sires treated as random in the separate analyses.

A maximum likelihood related multi-trait procedure was used to obtain variance and covariance estimates. Cows that had age and weight at calving measures did not necessarily have reproduction measures, and this method allows for sequential culling of records.

The model for each trait was:

$$Y_{ijklm} = \quad + hys_i + m_j + g_k$$

$$+ s_{kl} + e_{ijklm}$$

where Y_{ijklm} is the ijklmth observation of the trait, is the population mean, hys_i is the fixed effect of the ith herd-year-season (HYS) of birth (HYS subclasses were formed as March 1 to August 31 and September 1 to February 28 with an average of approximately four and three records per HYS for Holsteins and Ayrshires, respectively), m_j is the fixed effect of the jth month of calving, g_k is the fixed effect of the kth sire group (random sires were allocated to one of seven groups based on the year of birth of first daughter and all fixed sires were assigned to an eighth group), s_{kl} is the effect of the lth sire within the kth group (proven sires were treated as fixed while sampling sires were considered as random), and e_{ijklm} is the random residual associated with the ijklmth cow. Data from the two breeds were analyzed separately.

Seven traits were included in the multi-trait analysis: AGE, BW, DFB, DO, SC, GL and DD. Paternal half-sib heritability estimates and genetic and phenotypic correlations were computed from the appropriate estimates of sire and residual variances and covariances.

RESULTS AND DISCUSSION

Means and standard deviations for the seven traits in Ayrshires and Holsteins are in Table 1. The average age at calving of 29.2 months for Ayrshires was one month older than in Holsteins. Holsteins were on average 53 kg heavier after calving than the Ayrshires. Less variation was observed for both age and weight in Ayrshires than in Holsteins.

Differences between the two breeds were small for measures of fertility. Ayrshires averaged 3 more DFB, 6 more DO and 0.09 more SC than Holsteins. Mean gestation length and number of days dry were very similar for Ayrshires and Holsteins.

Heritability

Heritabilities of the traits are in Table 2. Values were similar for the two breeds, although slightly lower for the Ayrshires for all traits. Heritabilities of cow fertility were all less than .015 in the multi-trait analyses. Body weight was the most heritable of the traits studied.

Correlations

Phenotypic correlations between the traits were almost identical for the two breeds and are in Tables 3 and 4 for Ayrshires and Holsteins, respectively. The measures of fertility, DFB, DO and SC were not correlated with either AGE, BW or GL. The positive correlations indicate that longer DFB and DO and more SC are associated with a longer dry period. The phenotypic correlations indicate that a longer interval to first breeding and more services per conception are associated with more days open.

Increased age at calving was associated phenotypically with heavier body weight at calving. The positive phenotypic correlation observed between these traits may be a result of advancing or delaying breeding to have the

cows calve at a certain time of the year. The fact that longer gestation lengths are associated with more days dry is understandable if dairymen are making decisions to aim for a certain length of dry period assuming an average length of gestation.

Genetic correlations were similar for Ayrshires and Holsteins (Tables 3 and 4) for most traits with the exception of correlations involving DFB and SC. All of the genetic correlations that were different in sign between the two breeds involved the traits DFB and SC. These two traits had heritabilities estimated to be less than .008 in Ayrshires.

Genetic correlations between DO and AGE and BW and GL were very small in both the Ayrshire and Holstein breeds. The genetic correlation between SC and BW of -.30 in Holsteins suggests that genetically heavier cows require fewer services to conceive during first lactation.

The moderately positive genetic correlations of .44 and .48 between DO and DD in Ayrshires and Holsteins, respectively, indicate that cows with poorer reproductive performance will have longer dry periods. This is supported by the genetic correlations between DD and both DFB and SC. Days dry was positively correlated with AGE and GL. There was a small negative genetic correlation between DD and BW in Holsteins, and this relationship was more pronounced in Ayrshires.

Estimates of the genetic correlation between age and weight at first calving were -.90 and -.68 in Ayrshires and Holsteins, respectively. Genetically, cows that are lighter in weight when they calve will be older. Breeding heifers according to size may contribute to the negative genetic correlation between BW and AGE. Faster growing animals would tend to be bred younger and to calve earlier.

CONCLUSIONS

This study indicates little difference between the Ayrshire and Holstein breeds for reproductive performance and its relationship to the other traits studied. The phenotypic performance of the Quebec population was similar to that previously reported by other researchers.

Heritability estimates and coefficients of genetic variation for fertility traits tended to be low. Including a number of fertility traits in a multi-trait analysis may partition the genetic variation for the reproductive complex into its different components. If this is true and given the positive correlation between measures of fertility, it may be useful to select one of the current measures available from field data to represent female fertility. Of the measures studied, DO would be preferred due to the lack of correlation between it and age and weight at calving in both breeds, a higher heritability and more variation than DFB, and the fact that it is more normally distributed than SC.

Finally, the genetic variation observed in the present study was based on sampling sires. It would be useful to examine whether there is more variation for fertility when proven sires contribute to the estimates of sire variance, which might occur if there is a negative correlation between production and fertility.

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TABLE 1. Means and standard deviations of the traits.

Trait	Ayrshire			Holstein		
	No. of records	Mean	SD	No. of records	Mean	SD
Age at calving (days)	7824	891	111	79755	860	149
Weight at calving (kg)	7824	450	47	79755	503	81
Days to first breeding	7214	82	24	73829	79	24
Days open	6213	107	47	63643	101	46
Services per conception	6213	1.67	1.04	63643	1.58	.94
Gestation length (days)	5561	282	5	56725	282	5
Days dry	5561	64	18	56725	62	17

TABLE 2. Heritabilities and coefficients of additive genetic variation (CV).

Trait	Ayrshire		Holstein			
	Multi-trait		Single trait[1]		Multi-trait	
	h^2	CV	h^2	CV	h^2	CV
Age at calving	.034	.9	.090	1.3	.039	.9
Weight at calving	.072	1.2	.177	1.7	.132	1.5
Days to first breeding	.003	.8	.010	1.4	.004	.9
Days open	.004	1.3	.039	4.3	.013	2.5
Services per conception	.008	2.5	.043	6.0	.013	3.3
Gestation length	.012	.1	.110	.3	.046	.2
Days dry	.005	.9	.040	2.6	.013	1.5

[1] All single trait estimates were based on 53,174 observations.

TABLE 3. Genetic and phenotypic correlations between first lactation traits of Ayrshires[1].

	1	2	3	4	5	6	7
1. Age at calving		-.90	.68	-.09	-.53	.83	.54
2. Weight at calving	.43		.77	-.10	.33	-.76	-.66
3. Days to first breeding	.02	-.03		.37	-.16	.54	.75
4. Days open	.03	-.01	.45		.79	.13	.44
5. Services per conception	.02	.01	-.05	.71		-.49	.07
6. Gestation length	-.10	.04	-.02	.00	.00		.50
7. Days dry	.02	.01	.16	.37	.28	.26	

[1] Genetic correlations above the diagonal and phenotypic correlations below.

TABLE 4. Genetic and phenotypic correlations between first lactation traits of Holsteins[1].

	1	2	3	4	5	6	7
1. Age at calving		-.68	-.26	-.06	.08	.58	.26
2. Weight at calving	.41		.12	-.07	-.30	-.16	-.10
3. Days to first breeding	.01	-.03		.60	.28	-.14	.54
4. Days open	.02	-.01	.45		.89	.05	.48
5. Services per conception	.02	.00	-.05	.73		.03	.31
6. Gestation length	-.08	.04	-.02	.00	.01		.58
7. Days dry	.02	.01	.16	.38	.29	.29	

[1] Genetic correlations above the diagonal and phenotypic correlations below.

RELATIONSHIPS AMONG GESTATION LENGTH, CALVING EASE AND CALF MORTALITY IN HOLSTEIN CATTLE

K. Nadarajah and E. B. Burnside
Centre for Genetic Improvement of Livestock, Department of Animal & Poultry Science, University of Guelph

SUMMARY

Records of calving data for the first three parities of Ontario Holstein cows for gestation length, calving ease and calf mortality were analyzed to estimate the phenotypic and genetic correlations among these traits. A restricted maximum likelihood (REML) procedure suitable for multivariate mixed model analyses was used to estimate the variance and covariance components among traits. Average gestation length was 281.3 $\pm$ 6.0 days. Incidence of calving difficulty in cows at first freshening was 20.2%, but below 9.0% in subsequent parities. Average calf mortality rate was 5.5% across all parities, with first parity losses approaching 10.0%. All three traits were influenced by parity and sex of calf. Heritability estimates obtained from service sire variance components for gestation length, calving ease and calf mortality were 0.27, 0.07 and 0.01, respectively. Genetic correlation between gestation length and calving ease was 0.27 and between gestation length and calf mortality was 0.59, which indicates that shorter gestation length means easier calving and reduced calf mortality. Accurate prediction of low and high risk bulls for calving difficulty would be helpful to keep calving problems at minimum levels.

INTRODUCTION

Difficult parturition in dairy cows begins with some calf losses but may be followed by low production and reduced fertility, (Price and Wiltbank, 1978; Djemali et al., 1987). Incidence of dystocia at first calving has been reported as 15 to 17% in Dutch Frisians (Meijering, 1980), and 19 to 25% in U.S. Holsteins (Djemali et al., 1987). Gestation length has been found to be phenotypically and genetically associated with calving ease (Philipsson, 1976; Meijering, 1985). While difficult calving was associated with longer gestation periods, Philipsson (1976) also observed the frequency of still births was high with either extreme of gestation length in Swedish dairy cattle. Culling of top producing young cows either for calving problems or for impaired reproductive performance could affect genetic progress for production traits. A thorough understanding of phenotypic and genetic relationships among gestation length, calving difficulty and calf mortality would be helpful in formulating selection programs since the literature does not offer a complete picture of the complex problem of calving difficulty.

The objectives of this research were to study the factors that influence gestation length, calving ease and calf mortality of Canadian Holstein cattle and to estimate genetic and phenotypic relationships among these traits. Results of this study would be useful to the dairy industry in formulating selection programs.

MATERIALS AND METHODS

Calving ease data (1981 to 1986), recorded by Canadian milk recording agencies and from breeding receipts obtained from the three major Ontario AI Centres (Eastern Breeders, United Breeders and Western Ontario Breeders) were matched to compute gestation length of Ontario Holstein cows. The computational procedure has been described in detail in a previous report (Nadarajah et al., 1988). Records of gestation length (GL), calving ease score (CE) and calf mortality status (CM) comprised of 66,064 calving records up to three parities were available for this study. All three traits were considered as traits of the calves, which were progeny of 511 AI sires. There were four categories of CE (1 = Unobserved or unassisted, 2 = easy pull, 3 = hard pull, and 4 = caesarian), and CM was recorded as either alive (1) or dead (0) within 48 hours of calving.

A restricted maximum likelihood (REML) procedure (Meyer, 1985), suitable for a multivariate mixed model was used to estimate variance and covariance components among traits. The design matrices were equal for all traits. The above procedure involves transformation of the data vector to a canonical scale, which effectively reduces the multivariate situation to three corresponding univariate analyses. Since two of the traits considered in

this analyses (CE and CM) have multinomial and binomial distributions respectively, transformation of the data vector should also improve the accuracy of the parameter estimates in comparison to using the raw data and other methods (Tong et al., 1977 and Gianola, 1979).

The mixed model describing records of the three traits with herd - years, parity and sex of calf as fixed effects and sire of calf and residual error as random effects is found in Figure 1.

In general, a few bulls among the AI sires are well noted for causing calving problems. Breeders tend to use these high risk bulls on older cows. Therefore, treating herd-year and parity, should eliminate some bias due to the non-random use of sires in herds on different parity cows. Incorporation of the inverse of the relationship matrix (A^{-1}) of bulls accounted for a total of 551 sires in these analyses. Covariance between sires and residuals was assumed to be zero. Starting values for the ratio of the sire and residual variance components were obtained from three separate univariate analyses using a similar basic model to the one described in Fig.1, and null ratios were assumed with respect to covariance among traits. In the multivariate analysis, a reasonable convergence was achieved after 20 rounds of iteration. Heritabilities, phenotypic and genetic correlations were computed from the corresponding estimates of variance and covariance components. Prediction of Best Linear Unbiased Prediction (BLUP) ratings of bulls for the respective traits was carried out using the final estimates of sire and residual variance/covariance components.

RESULTS AND DISCUSSION

Distribution of records over the four CE categories and the associated means for GL are shown in Table 1. Overall, 57.3% of the calvings were unobserved or unassisted, 32.3% were normal (easy pull), 10.0% were hard pull (difficult), and veterinarian intervention accounted for only 0.4%. The average GE for unassisted calvings was about two days shorter than the mean GL for the difficult (hard pull) calvings. Although the incidence of caesarians was very low, this category was associated with greater variation in length of gestation. The GL was found to increase with parity, whereas calving problems and calf mortality rate decreased with parity (Table 2). First calvers experienced almost 20% calving difficulty (hard pull plus caesarian) compared with 8.2 and 7.3% for the second and third parity cows. Djemali et al., (1987) reported the incidence of dystocia in U.S. Holsteins at first calving as 19 to 25% using calving data from the Mid Western States DHIA and National Association of Animal Breeders.

In spite of the selective usage of low risk bulls on heifers by breeders, first parity cows experienced 0.66% caesarian cases which was about a three fold increase over the incidence of caesarian cases observed in second and third parity cows in these data. Mean gestation length for Holstein cows was 281.9 ± 5.9 days and the average mortality rate of calves was 5.48%. Calf mortality rates for first parity cows was over 10%, and possibly quite a large percentage of calf losses could have been directly attributed to calving problems.

Gestation length seems to vary among breeds. In a review paper Anderson and Plum (1965) reported a weighted average of 280 days for Holstein cows. Several researchers found that GL was influenced positively by age of dam.

In this study, GL was about a day shorter for heifer calves compared to bull calves, and calving problems and CM rates were slightly higher for male calves. This may be attributed to the size of the calf and it's association with longer GL. Using a similar data set, Nadarajah et al.,(1988), found that medium and large Holstein calves were associated with extended gestation lengths of 1.7 and 3.6 days, respectively.

The REML estimates of sire and residual (co)variance components for the three traits are given in Table 4, and corresponding estimates of heritabilities, phenotypic and genetic correlations are shown in Table 5. Estimates of service sire variance components for GL, CE and CM were 2.25, 0.006 and 0.0002, respectively, and corresponding heritability estimates were 0.27, 0.07 and 0.01 respectively.

In a previous study, applying a sire and maternal grand-sire model analysis (Nadarajah et al., 1988), it was observed that GL was more dependent on the service sire compared to the influence of maternal grand-sire through the dam of the calf. From the Mid-west dairy cooperatives calving data, Pollak and Freeman (1976) reported a heritability estimate of 0.06 for calving difficulty, while with more additional data and incorporation of the relationship matrix, Berger and Freeman (1978) reported the following heritability estimates for calving difficulty : 0.17 $\pm$ .05 for first calf heifers, 0.08 $\pm$ .04 for second and 0.05 $\pm$ .02 for third and later parity cows with a pooled estimate of 0.08 $\pm$ .04. In a recent study, Meijering (1985) reported heritability estimates for GL, dystocia score and stillbirth as 0.458, 0.093 and 0.003, respectively.

The genetic correlation between GL with CE was 0.27, and between GL and CM was 0.59, while genetic correlation between CE and CM was 0.16. Phenotypic correlations were slightly lower than genetic correlations. This might be due to the mating strategy followed by individual breeders in their herds. Estimates of heritabilities or correlations from these data could be an underestimate because of the selective use of low risk bulls on heifers and high risk bulls on older cows. Therefore, the true genetic variation among bulls might be greater under a strict random mating situation.

A moderate heritability for GL indicates that selection of sires to reduce length of gestation is practical. The direct relationships between GL, CE and CM in this investigation also indicate that selection for shorter GL would improve ease of calving and reduce calf mortality, which in turn should increase the lifetime reproductive performance of cows. Philipsson (1976) suggested that selection for shorter gestation length may lead to higher incidence of still births. This study suggests the opposite, since the genetic correlation between GL and CM was + 0.59, indicating that a shorter GL means less calf mortality. It may be wise to record GL and select sires to reduce it slightly, especially in breeds that experience significant calving losses. The other alternative would be to continue to identify low and high risk bulls and follow the present mating strategy to keep calving problems at minimum levels. This procedure has the advantage of removing GL from the selection goal.

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Figure 1. Mixed model for gestation length, calving ease and calf mortality

$$Y_i = X_{1i}h_i + X_{2i}b_i + X_{3i}c_i + Z_iu_i + e_i \qquad (i=1,2,3)$$

with

$$E(Y_i) = X_{1i}h_i + X_{2i}b_i + X_{3i}c_i,\ E(u_i) = E(e_i) = 0,$$

$$Var(u_i) = G_{ii} + A^{-1},\ Var(e_i) = R_{ii},\ Cov(u_i,e_i) = 0$$

and

$$Var(Y_i) = V_{ii} = Z_i(G_{ii}+A^{-1})Z_i' + R_{ii}$$

where

Y_i = a vector of observations (GL, CE, CM) of lengths = 66064,

X_{1i} = a known matrix relating herd-year effects to observations,

h_i = an unknown vector of fixed herd-year effects (7887 levels),

X_{2i} = a known matrix relating parity effects to observations,

b_i = an unknown vector of fixed parity effects (1, 2, or 3),

X_{3i} = a known matrix relating sex of calf effects to observations,

c_i = an unknown vector of fixed sex of calf effects (M or F),

Z_i = a known matrix relating sire of calf effects to observations,

u_i = an unknown vector of random sire of calf effects (551 bulls),

e_i = an unknown vector of random residuals (66,064 levels).

G_{ii} = variance covariance matrix, and

A^{-1} = inverse of the numerator relationship matrix among bulls.

Table 1. Frequency distribution of calving ease and associated length of gestation (GL) in Holstein cows.

Calving Ease Categories	No. of Calvings	GL + SD	C.V.
1. unobserved or unassisted	37,887	281.6 ± 5.9	2.1
2. Easy pull (normal)	21,354	282.1 ± 5.8	2.1
3. Hard pull (difficult)	6,628	283.1 ± 5.9	2.1
4. Caesarian or Veterinarian Intervention	195	282.3 ± 7.3	2.6

Table 2. Influence of parity and sex (the estimates of difference) on gestation length (GL), calving ease (CE) and calf mortality (CM).

Factors	N	GL (in days)	CE (lin. score)	CM (bin.)
Parity:				
1	13,392	0.00	0.00	0.00
2	19,028	0.95	-0.06	-0.30
3	33,644	1.42	-0.55	-0.33
Sex:				
M	32,250	0.00	0.00	0.00
F	32,814	-0.83	-0.01	-0.13

Table 3. REML - estimates of sire[1] and residual variance - covariance components for GL, CE, and CM.

	GL	CE	CM
GL	2.2535 30.9203	0.0313 0.2841	0.0031 -0.0028
CE		0.0062 0.3733	0.0006 0.0219
CM			0.0002 0.0501

1. sire variance - covariance estimates appears in the first row.

Table 4. Estimates of heritabilities (on the diagonal), phenotypic (above diagonal) and genetic (below diagonal) correlations for GL, CE and CM.

	GL	CE	CM
GL	0.27	0.09	0.00
CE	0.27	0.07	0.16
CM	0.59	0.16	0.01

REGIONAL HETEROGENEITY OF VARIANCES AND ITS EFFECT ON CANADIAN HOLSTEIN SIRE EVALUATION

P.G. Sullivan and L.R. Schaeffer
Centre for Genetic Improvement of Livestock, Department of Animal & Poultry Science, University of Guelph

INTRODUCTION

Artificial insemination (AI) studs in Canada serve distinct geographical areas and dairy bulls are used primarily within AI stud regions. Selection practices over time may have varied among regions and population means and variances are known to change as a result of selection. Thus, regional heterogeneity of population structure may exist in Canada. Some of the problems in sire evaluation resulting from heterogeneity caused by selection have likely been accounted for by incorporating genetic relationships among sires in the mixed model equations. Random chance differences in the base population structures of each region, or an incomplete accounting of selection, however, may still cause problems with sire evaluations.

Grouping strategies have been examined which account for regional differences in genetic mean, and have been shown to be relatively ineffective when semen exchange between regions is limited. Regional independence, while making genetic grouping ineffective, would not pose a problem when accounting for differences in regional variances.

The purpose of this study was to explore the possible existence of regional differences in genetic and phenotypic variances and to examine the potential impact of heterogeneous variances on Holstein sire evaluation for the production traits of milk and fat yield and the type conformation trait of final class score.

MATERIALS AND METHODS

All first lactation production records used to evaluate Canadian dairy sires in the spring of 1987 were obtained from Agriculture Canada. There were 1,426,340 records, of which 948,413 were made by Holsteins in Ontario and Quebec. Some of the records in Ontario were eliminated because of problems identifying the AI stud area in which they were made, leaving 880,329 records to be used in this study. Milk and fat yields were the traits of interest and records were expressed as Breed Class Averages (BCA), preadjusted for the effects of age and month of calving and to a lactation length of 305 days. The earliest records in the data were collected in 1958.

For overall conformation, 441,230 final class records from Ontario and Quebec were obtained from the University of Guelph, which were part of the Spring 1987 sire evaluation run. Final class records, which were categorical and ranged from 1 to 18, were transformed to final class score (range 0 - 100). The most recent records on each cow, in contrast to the first lactation records for production, were utilized. The earliest type records in the data were made in 1981.

The data for each trait were divided regionally, each region served by a different AI stud. Quebec represented one region, while Ontario was divided into the Eastern, Central/Northern, and Western regions which are served by the three major AI studs in that province. Semen from a limited number of bulls is now available to breeders in each region at equal cost, but for the majority of bulls there is financial incentive to purchase semen within region.

The model of analysis for the traits of milk and fat yield included the fixed effects of herd-year-season and genetic group of the sire, groupings by year of entry into AI for AI bulls and by registration number for non-AI bulls. In order to minimize the amount of selection bias in the variance component estimates, sire effects were treated as fixed if records made by daughters that were conceived during the sire's sampling period were missing from the data. The model for final class score included fixed sire effects, as with the production traits.

Sire and maternal grandsire relationships were included in the analyses for all three traits. Variance components were estimated within each region by both the pseudo expectation (PE) approach and the

tilde-hat (TH) approach, incorporating the inverse of the numerator relationship matrix into the quadratic forms of each method.

Sires were evaluated within and across regions either assuming or ignoring regional heterogeneity of variances. In the heterogeneous case, the ratio of the regional variance component estimates from this study were assumed for each region. In the homogeneous case, the average of the regionally estimated ratios, weighted by the number of effective records in each region, was assumed. Additionally, sires were evaluated within each region assuming variance ratios that had been used in the most recent (Spring, 1987) national sire evaluations for each of the three traits. Heritabilities associated with these ratios were 0.25 for the yield traits and 0.18 for final class score.

When regional heterogeneity was assumed and sires were evaluated across regions, the variance-covariance matrix (G) of random sire effects was not equal to the numerator relationship matrix times a constant sire variance, so the method of Elzo (1986) was used to compute the required G^{-1} for those evaluations.

Bulls with accurate ($r_{TI} > .75$) regional proofs (evaluations) in more than one region were chosen to examine the effect of the assumed variance ratios on the regional consistency of sire evaluation. The selected proofs were representative of publishable Canadian proofs which have a minimum accuracy of .742.

Pedigree indices were computed as half the sire's proof plus one quarter of the maternal grandsire's proof. The correlation between the proofs and pedigree indices of these bulls was used to determine which method of evaluation resulted in the most consistency between a sire's proof and pedigree index.

RESULTS AND DISCUSSION

The amount of semen exchange among AI stud regions, as exemplified in Table 1, has been limited. More than 80% of the semen use in Ontario and Quebec has been within region and less than 5% of the bulls used have had daughters or granddaughters in all four regions.

Heritability estimates using both PE and TH are in Table 2. PE estimated heritabilities suggested that regional heterogeneity existed for all three traits. The same conclusion might have been drawn from the TH production trait estimates, although regional heterogeneity was less evident with the TH estimates for final class score. Differences between the methods, PE and TH, were as large as differences between the regions. The hypothesis of regional homogeneity of variances and heritabilities could therefore not be rejected.

Sires ranked virtually the same when accounting for regional heterogeneity of variances as when homogeneity was assumed. Rank correlations between proofs on all sires and sires with multiple region proofs, assuming, versus not assuming, homogeneous variances, were near unity for all traits (Table 3). Regional heterogeneity of variances can likely be ignored without seriously affecting national Holstein sire rankings for milk yield, fat yield or final class.

Correlations were lower for final class score than for the yield traits, suggesting that regional inconsistencies were more prominent for this trait. Lower correlations were expected for final class score because of a lower heritability, but the average accuracy of publishable proofs was only slightly lower than for the yield traits (.86 versus .88).

Correlations between a sire's proof and his pedigree index (Table 4) were fairly robust to changes in the assumed variance ratios, although slightly less so than the correlations between regional proofs above.

Regional heterogeneity of variances is likely of minor importance to national sire evaluation. Sire rankings would not be seriously affected, even if heterogeneity of variances was substantial. Many of the estimates from this study suggested that heterogeneity existed, but errors in the estimates were larger than was expected due to sampling. Selection bias was presumably responsible for much of the additional error in the estimates. For all three traits, use of the ratio that had been assumed in the most recent national evaluations resulted in more

consistency between regional proofs of a sire and between a sire's proof and pedigree index than did use of either the PE or the TH estimates of this study.

Table 1. Number (and percentage) of sires whose descendants are either in all four regions or are restricted to one region

Minimum # of Daughters Per Region in:	Descendants in: One Region	Four Regions	Total
(Production Data)			
0	21660 (80%)	1033 (4%)	26955
20	2470 (87%)	93 (3%)	2849
50	1160 (85%)	45 (3%)	1363
(Type Data)			
0	9103 (80%)	511 (4%)	11428
20	1126 (87%)	37 (3%)	1299
50	581 (89%)	14 (2%)	652

Table 2. Heritability estimates, by region, for the traits of milk yield, fat yield and final class score.

Region	Number of Records*	VC+	Trait: Milk Yield h^2	Milk Yield SE**	Fat Yield h^2	Fat Yield SE**	Final Class Score h^2	Final Class Score SE**
Western Ontario	168,868 / 68,224	PE	0.320	0.010	0.527	0.013	0.327	0.016
		TH	0.451	0.012	0.407	0.012	0.181	0.013
Central/ North. Ont.	271,909 / 126,418	PE	0.370	0.008	0.382	0.008	0.337	0.012
		TH	0.386	0.008	0.358	0.008	0.182	0.009
Eastern	147,316 / 80,841	PE	0.328	0.011	0.309	0.011	0.196	0.012
		TH	0.351	0.011	0.325	0.011	0.166	0.012
Quebec	292,236 / 165,747	PE	0.459	0.008	0.363	0.007	0.473	0.011
		TH	0.331	0.007	0.349	0.007	0.222	0.008

* - records for: yield traits / final class score.
\+ - estimation procedure used: PE = pseudo expectation; TH = tilde hat.
** - Henderson's Method 1 approximation, from Swiger et al. (1964), using effective numbers in place of actual numbers of records.

Table 3. Rank correlations of across region sire proofs for milk yield, fat yield and final class score, with versus without the assumption of homogeneous regional variances.

Trait		# of sires	Variance estimates used: PE[+]	tilde-hat
milk yield	all sires	26,955	0.996	0.998
	m.r. sires*	5,295	0.996	0.998
fat yield	all sires	26,955	0.991	0.999
	m.r. sires*	5,295	0.994	0.999
final class score	all sires	11,428	0.989	0.998
	m.r. sires*	2,325	0.985	0.998

[+] - pseudo expectation
* - sires with multiple region proofs

Table 4. Correlations between milk yield proofs and pedigree indexes.

Region	# of sires	Milk	Fat	Final Score
Western Ontario	108	.535	.653	.328
Central/ North. Ont.	144	.585	.678	.451
Eastern Ontario	87	.609	.765	.633
Quebec	168	.569	.670	.505

SELECTION OF SIRES ORIGINATING FROM A NUCLEUS BREEDING UNIT FOR USE IN A COMMERCIAL DAIRY POPULATION

G. Teepker[1] and D.S. Keller
Centre for Genetic Improvement of Livestock, Department of Animal and Poultry Science, University of Guelph and [1]Institute for Animal Breeding and Genetics, University of Goettingen

SUMMARY

Bulls that originate from breeding schemes with extensive use of multiple ovulation and embryo transfer (MOET) can be selected on pedigree information, the performance of their contemporary female full and half-sibs or based on a progeny test. Combined selection across groups of bulls with different sources of information is shown to be superior to selection of progeny tested bulls alone. The magnitude of the superiority for a given selection rate is determined by the differences in genetic merit between the groups and ranges from 5 to 65%. Depending on the annual genetic progress the majority of the bulls are selected either on pedigree or progeny information. The proportion of sib-tested bulls ranges from 9 to 23%. When breeding activities are concentrated in nucleus herds, possible genotype by environment interactions between performance in the test herd and in the commercial population have to be considered. Genotype by environment interactions increase the proportion of progeny tested sires that will be selected. However, the proportion of progeny tested sires used is reduced by genotype by environment interactions among the groups of a heterogenous commercial production environment.

INTRODUCTION

The use of multiple ovulation and embryo transfer (MOET) for the improvement of dairy cattle populations has been investigated in several studies, recently reviewed by Ruane (1988). With reproductive techniques like MOET becoming available, concentration of breeding and selection activities in a relatively small nucleus herd has been shown to increase annual genetic response. Sires born in the nucleus herd can be used to transmit the genetic progress to the commercial population. Different methods of testing these sires may be considered. In a juvenile MOET scheme (Nicholas and Smith, 1983) high annual genetic response is obtained by selecting young bulls (YB) only based on their pedigree information at one year of age. In an adult MOET breeding scheme (Nicholas and Smith, 1983) information on the performance of contemporary full-sibs and half-sibs will be available for selection of sib-tested bulls (SB) at an age of about 3 years. The most accurate selection would obviously be based on a progeny test (PB) with the information on the daughters available after about another 2 - 4 years. However, the increased generation interval with progeny testing can more than offset the increased accuracy leading to lower rates of annual genetic response.

The objective of this study is to compare the efficiency of alternative methods of selecting bulls from a nucleus herd for commercial use. In contrast to the work by Smith and Ruane (1987), who calculated the merit of bulls selected on progeny information and sib information in a traditional breeding program, breeding in this study is done in a closed nucleus herd. Testing other than progeny test is also assumed to be done within one single herd. Differences due to genotype by environment interactions (GxE) between the specific environment (nucleus herd) and commercial conditions may lower the efficiency of selection. Thus GxE between the 'test station' and the performance of the offspring under different commercial environments are considered. Under heterogenous commercial conditions GxE may also exist between the different production levels within the commercial population. This would affect the response of a progeny testing system.

METHODS

The approach in this study was adapted from the paper by Smith and Ruane (1987) and extended for the case of selection between animals of three groups. A more general algorithm to select across groups (generations) of animals with different distributions was recently given by Ducrocq and Quaas (1988).

Assume a juvenile MOET nucleus breeding scheme for dairy cattle (Nicholas and Smith, 1983) with some modifications. Semen of all young bulls prior to selection is collected and stored for later use if sib-selected. All females are performance tested, even if they are not selected according to their pedigree index. Thus, sib information for each young bull will be available. Also, all young bulls are assumed to be progeny tested in the commercial population. Let the distribution of estimated breeding values (EBV) for PB, SB and YB be characterized by their means m_1, m_2, m_3 and the corresponding standard deviations s_1, s_2 and s_3. If there is a positive genetic trend in the nucleus breeding herd, the average merit of YB should be higher than the mean of the SB and higher still than the mean of the PB. Depending on the accuracy of evaluation, the EBV's are regressed to the mean of the groups, giving a larger standard deviation of the EBV's for PB than for SB and YB, respectively. The phenotypic standard deviation (sd) is set as 1.0 with a heritability of h^2=.25.

The three groups of bulls are assumed to be of the same size. Only one male per full-sib family is included to allow selection of individuals (rather than families), based on pedigree index and sib-test, respectively. Alternatively, distinction between full-sib males could be made by ranking individuals within a full-sibship by using indicator traits (Woolliams and Smith, 1988). A given number of bulls must be selected for commercial use. These can be selected from within one group or, depending on their EBV's, across the groups of PB, SB and YB. The common threshold point T for selection across groups (for $m_1 = 0$) can be found such that

$$T = x_1 s_1$$

$$T = m_2 + x_2 s_2$$

$$T = m_3 + x_3 s_3$$

for $p' = p_1 + p_2 + p_3$

where p' is the given proportion to select overall and p_1, p_2 and p_3 are the proportions selected from each group with corresponding normal deviates x_1, x_2 and x_3. The common threshold point T is found iteratively by a computer program using normal distribution tables.

The overall mean of the selected sires is calculated as

$$M_2 = \frac{p_1 a_1 s_1 + p_2(m_2 + a_2 s_2) + p_3(m_3 + a_3 s_3)}{p_1 + p_2 + p_3}$$

where a_1, a_2 and a_3 correspond to the standard selection differential of the selected proportions p_1, p_2 and p_3. The overall response of the combined selection is compared to the response when selection is based only on progeny testing (M_1) with $M_1 = a' s_1$, where a' is the selection differential when a proportion p' is selected (p' set to 10 percent). Ignoring possible genotype by environment interactions, the annual genetic response in the breeding unit and the time between groups determine the mean of the groups. The time difference between YB and SB is assumed to be 2 years, the difference betwenn YB and PB is varied from 4 to 6 years to allow for different progeny test program efficiency. The annual genetic trend in the nucleus herd is .05, .10 .15 sd, representing a poor, medium and highly efficient breeding program. Theoretical rates of response in the range of .10 to .20 sd per year were calculated by Nicholas and Smith (1983) for adult and juvenile MOET nucleus schemes. Breeding value estimation is based on 50 daughter records for PB and 7 female full-sibs for SB. The pedigree index for YB includes 7 female full-sibs of the sire, one record on the dam and 6 full-sibs of the dam. In the case of no GxE the accuracies are then .88 for PB, .50 for SB and .40 for YB. Alternative mating structures in the nucleus with fewer full-sibs but additional half-sibs would provide equivalent accuracies. The amount of genetic information is cumulative as a bull ages. However, parental information used to evaluate YB is expected to contribute little to selection of SB and PB; similarly additional sib-information increases the accuracy of sire proofs for PB only marginally. Therefore, these information sources are not utilized.

Genotype by Environment Interactions

Genotype by environment interaction

can be expressed as the genetic correlation between the genotypic merit of an animal in different environments (Falconer, 1952). A genetic correlation significantly less than 1.0 indicates a GxE interaction.

Following Merks (1988), a schematic desciption of the GxE problem for a dairy cattle situation is given in Figure 1. The nucleus unit is assumed to have one homogenous production environment. The correlation r_{tc} represents the genotype - environment correlation between the performance in the test herd and in an average commercial herd. The value of the correlation r_{tc} is varied from 1.0 to .5. The GxE among the commercial herds involving a variety of production systems and conditions is given by the correlation r_{cc}. Two extreme values are used for this correlation, setting r_{cc} either to 1.0 or equal to r_{tc}. This implies the assumption that the genetic correlation (r_{tc}) between test herd and an average commercial herd can be seen as the lower limit for the genetic correlation among different levels of the commercial population (Merks, 1988).

Genotype by environment interactions reduce the standard deviations of the estimated breeding values of the sires, as shown in the Appendix. The means of the YB and SB are reduced by the factor r_{tc}, since the correlated genetic response in the commercial population and not the genetic trend in the nucleus determines the differences in genetic merit between the sires in the three groups.

RESULTS

No Genotype by Environment Interaction

The superiority of a combined use of YB, SB and PB compared to the selection within PB only is shown in Table 1. For a proportion of 10% selected and a genotype - environment correlation of 1.0 (no interaction), using sires selected across groups yields a superiority of 5 to 65 percent over using progeny tested sires alone. High annual genetic progress and long time lags until the progeny test results are available increase the relative mean of the YB and SB and the superiority of a combined selection. If the genetic trend is low (.05 sd/year), the chance for YB and SB to be selected is relatively low; 55 to 71 percent of the selected sires are PB (Figure 2a-c). The remaining proportion is equally divided between YB and SB. In turn, with high genetic progress (.15 sd /year) in the nucleus herd, most of the young bulls will be superior to the older but more accurately tested SB or PB. Therefore, between 71 and 85% of the selected bulls are YB. The predominance of YB use was also reported by Meuwissen (1988) for the optimisation of a dairy breeding scheme, where the optimum response of .158 sd per year was found by selecting young bulls only. Smith and Ruane (1987) showed a similar superiority of a combined selection of SB and YB. However, including YB for selection in the present study reduces the proportion of the selected bulls which are SB to 9 - 23%. Under the assumptions made in this study, the majority of the selected bulls are likely to be either YB or PB. The increased accuracy of sib information is not sufficient to counterbalance the additional two years time lag compared to YB when the genetic trend is high. In turn, for a low genetic trend, the high accuracy of a progeny test will outweigh the advantage of an earlier use of SB compared to PB. A higher overall selection rate would increase the proportion of YB and SB that are selected and the relative superiority of a combined selection across bull groups, as can be derived from the findings of Smith and Ruane (1987).

Genotype by Environment Interactions Between Test Herd and Commercial Population

Imposing a GxE between test herd and commercial herds (assuming no GxE within the commercial population), reduces the standard deviations of the commercial EBV's for YB and SB. Additionally, the difference in the means of the bull groups is diminished because only a correlated genetic response is transferred from the nucleus to the commercial population. Thus, a generally smaller proportion of YB and SB is likely to be selected (Figures 2a-c, for r_{cc} = 1.0). The superiority of selection across groups is also reduced (Table 1, for r_{cc} = 1.0). For a genetic trend of .10 sd combined selection seems to be worthwhile for r_{tc} greater than .6. Results for selection rates higher than 10% continue to show advantages of combined selection with even lower correlations (not shown).

Genotype by Environment Interactions Between Test Herd and Commercial Population As Well As Within The Commercial Population

The average GxE between different units within the commercial population was set equal to the GxE between test herd and an average commercial herd. Thus the commercial population is subdivided into a wide variety of commercial environments to which different genotypes respond differently. The standard deviation of the EBV's of the PB is reduced, as can be seen from formula (2) in the Appendix. The advantages of a progeny test as shown before, are partly reduced. The effect of GxE on the YB and SB is, however, always greater than on the PB, so that the superiority of selection across groups declines with lower genotype - environment correlations (Table 1, for $r_{cc} = r_{tc}$). The proportion of YB and SB that will be selected is decreasing linearily with greater GxE, but combined selection across groups of bulls leads to considerably higher merit of the selected bulls, even with fairly high GxE effects.

DISCUSSION

During the last few years the first nucleus breeding units for dairy cattle have been established in UK, France and Denmark. Major reasons for replacing traditional breeding programs involving a large part of the population are the predicted faster rates of genetic improvement with MOET systems and the use of a standardized environment for testing and selecting the breeding stock. Centralized testing and selecting would avoid preferential treatment of potential bull dams which can be a major factor reducing genetic progress in traditional breeding programs.

Standardization of the environment through the use of nucleus breeding programs may cause problems in the dissemination of the genetic merit from animals evaluated on the basis of nucleus test results, to the commercial population with a wide range of production conditions. Moderate GxE between central test results and the performance of progeny in commercial herds are reported in pigs by Merks (1988). Dickerson's (1962) classical paper examined genotype by environment interactions in poultry and reported important shifts in ranking of genetic stocks. For beef cattle (reviewed by Parnell et al., 1986) and sheep (Waldron et al., 1986) poor associations between station and on-farm performance are found, but true genotype by environment interactions are not clearly proved. Fairly low genetic correlations between dairy sire proofs from progeny test stations and tests in commercial herds are reported from the Danish testing stations in the 1950's. However, Touchberry et al. (1960) suggested that the lack of similarity of proofs was mainly due to the design of the station test rather than large interactions between heredity and environment.

A nucleus dairy cattle breeding unit will provide sires for artificial insemination in the commercial population. In an adult MOET nucleus breeding scheme, the parents and also the female sibs of the young bulls are tested under a specific environment which may be characterized by certain parameters: the rearing intensity for heifers, feeding intensity during lactation, use of production promotors such as somatotropin and special health care. For the commercial population, a variety of management levels generally exists. Additionally, milk quota restrictions may generate alternative breeding goals leading to another source of GxE interactions. For a dairy farm with no alternative use for its natural pasture it may, for example, be economically best to produce the quota limited milk without concentrates but by keeping more cows. The potential impact of resource constraints like a market share quota on ranking of dairy sires was illustrated by Sivarajasingam et al. (1984) using linear programming techniques.

Research is neccessary to match the enviromental conditions in the test and commercial environment, but not all differences can be specified. On the other hand, matching the environmental conditions in the nucleus unit and the commercial herds may not be feasible or even desirable. For the use of MOET schemes for faster genetic improvement of livestock in developing countries (Smith, 1988), severe differences between the two types of production environments seem to be almost inevitable to ensure normal MOET success rates in the breeding unit. Genotype by environment interactions may then be a major problem for transmitting the genetic response from a high level nucleus breeding unit to often

marginal conditions in the commercial production.

Regardless of their source, GxE will influence decisions on testing and selecting of bulls bred by nucleus units for commercial use. Selection of sires based on their EBV's and regardless of accuracy leads to maximum genetic progress, as emphasized by Smith and Ruane (1987). This was clearly demonstrated for the selection of sires across the groups of YB, SB and PB in the present study. Assuming that EBV's for YB, SB and PB are completely comparable, i.e. estimated under an Animal Model (Kennedy et al., 1988), selection across groups effectively increases the number of bulls available for selection (Smith and Ruane, 1987). Depending on the difference between the groups in genetic merit and the accuracy of estimation of breeding values, selection across groups yields a considerable superiority over selecting progeny tested sires only. The contributions of sib-tested bulls to this superiority are small, because either YB or PB have higher EBV's and ought to be selected. In practice, with juvenile MOET schemes one would have the option to select YB or PB for commercial use, because the assumption that sib-test results will be obtained for each bull may be irrelevant. Since the selection in MOET nucleus schemes is usually not very intense and the accuracy is relatively low, the accuracy of selecting YB is only marginally reduced due to reduced variation among their parents. Thus effective selection among YB is possible. An adult MOET scheme could provide YB and SB for the commercial population, which would subsequently be progeny tested. Rather than strict adherence to a juvenile or adult scheme, as proposed by Nicholas and Smith (1983), a nucleus unit should itself select sires with the highest EBV across bull groups for faster genetic improvement. By selection on EBV among all available bulls, the genetic gain is optimized as well as the generation interval (Meuwissen, 1988). The use of superior progeny tested bulls or even bulls from a foreign population as nucleus sires may be especially important in the establishment of a nucleus breeding scheme (Colleau, 1986). A progeny test of nucleus born sires would also be more advantageous for traits with low heritabilities (Christensen and Liboriussen, 1986; Teepker and Smith, 1989). However, if breeding activities are concentrated in a MOET nucleus herd economic constraints could make progeny testing infeasible. This would limit the selection decisions to the groups of YB and/or SB. The greatest reductions in the average EBV of the selected sires would be expected if only a poor genetic response is realized within the nucleus and an efficient progeny test (short time lag) has been discontinued.

Genotype by environment interactions between the evaluations in a single nucleus herd and the performance in the commercial population are also a strong argument to maintain widespread progeny testing. Progeny test results reflect more directly the genetic merit of a sire under a variety of enviromental conditions, even if the progeny testing may also be effected by GxE within the commercial population. However, higher heritabilities of traits measured under the defined conditions of a nucleus herd or additional indicator traits (Woolliams and Smith, 1988) may increase the accuracy of evaluating YB and SB and compensate for the loss in efficiency due to genotype by environment interactions. While the effect of GxE within the commercial population can be minimized by a widespread, well designed progeny test, the number of female full-sibs and half-sibs born in the nucleus herd is generally too small to test them over a sufficient variety of environments. Clones of these animals could be helpful in this case. If genotype by environment interactions are shown to be severe, special breeding programs for special environments are likely to be most efficient.

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APPENDIX

Standard deviations of estimated breeding values (EBV) in the case of genotype by environment interactions

The aggregate genotype (H) is defined as the overall economic merit of a sire's progeny for an average commercial herd. Estimated breeding values are calculated using selection index theory. The genetic parameters are assumed to be equal for the different levels (test herd and commercial herds) of the breeding program.

Selection of SB and YB in the test herd for use in commercial herds

Assume n full-sibs each with one record for each sib-tested bull. The variance of this information (P_n) is given by

$$var(P_n) = 1/n * [1 + (n-1).5\ h^2] * \sigma^2_P$$

with h^2 for the heritability and σ^2_P for the phenotypic variance of the trait. For a genetic correlation r_{tc} between the performance in the test herd and an average commercial herd, $cov(P_nH) = .5\ r_{tc}\ h^2\ \sigma^2_P$.

Following selection index procedures the standard deviation (SD_{EBV}) of the EBV's becomes

$$SD_{EBV} = h * \sigma_P * [\ (.25\ n\ h^2\ r_{tc}^2) / (1+(n-1).5\ h^2)\ ]^{**}.5 \qquad (1)$$

which can be rewritten as $SD_{EBV} = r_{tc} * R * h * \sigma_P$, where R is the accuracy of the evaluation for r_{tc} = 1.0. Similarily, the standard deviations of the EBV's for YB are also reduced by the factor r_{tc}.

Selection of progeny tested bulls (PB)

Assume that n daughters of each sire are tested in n different commercial herds. The genetic correlation between the performance of daughters of the same sire in different herds is given by r_{cc}, and contributes to the covariance between the half-sib progeny of a sire. The variance of the daughter mean then is

$$var(P_n) = 1/n * [1+(n-1).25\ h^2\ r_{cc}] * \sigma^2_P$$

The covariance between index information and the sire's genotype (for large n) becomes approximately $cov(P_nH) = .5\ r_{cc}\ h^2\ \sigma^2_P$. The standard deviation of EBV's (SD_{EBV}) can then be calculated as

$$SD_{EBV} = h * \sigma_P * [\ .25\ n\ h^2\ r_{cc}^2 / (1+(n-1).25\ h^2\ r_{cc})\ ]^{**}.5 \qquad (2)$$

Table 1: Relative genetic superiority (%) of bulls selected across groups[1] compared to use of progeny tested bulls alone (10% selection rate).

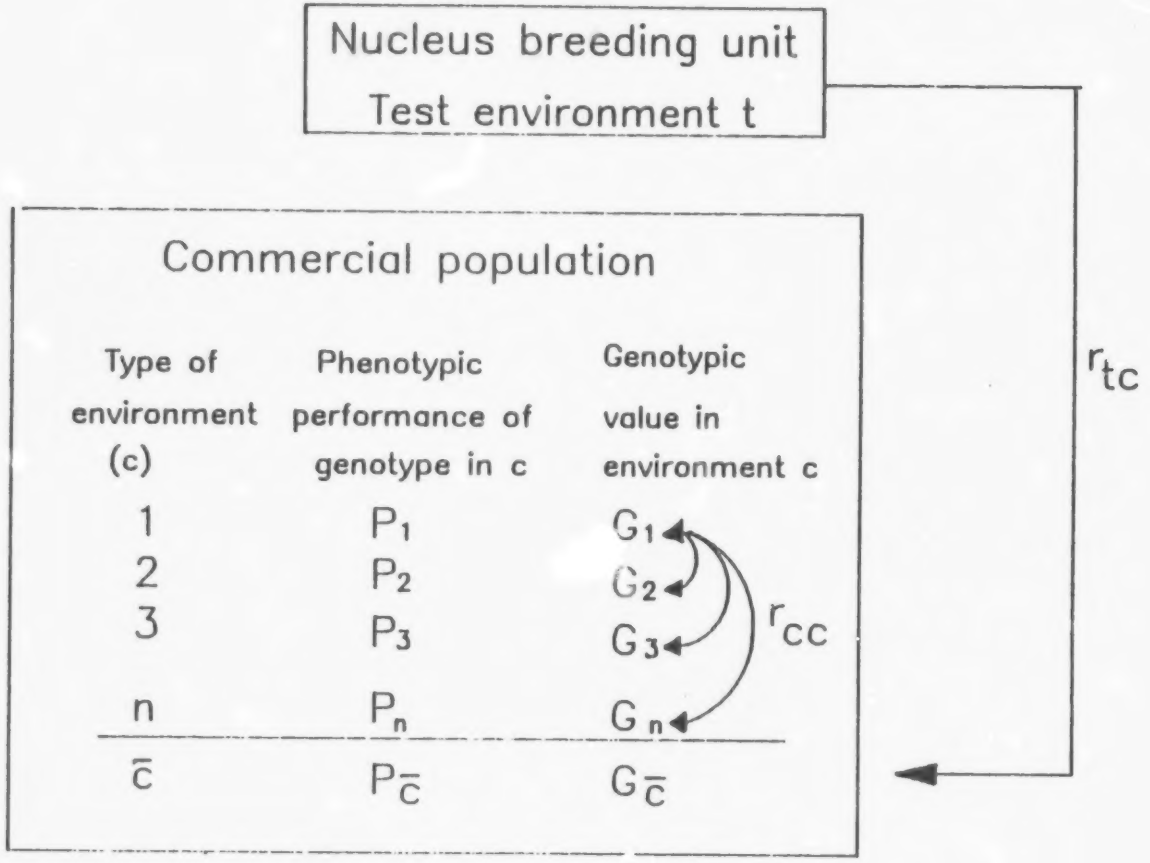
Genetic trend[2]	Years[3] PB-YB	GxE within commercial population (r_{cc})											
		$r_{cc} = 1.0$						$r_{cc} = r_{tc}$					
		GxE between test herd and commercial population (r_{tc})											
		1.0	.9	.8	.7	.6	.5	1.0	.9	.8	.7	.6	.5
.05	4	5	2	1	0	0	0	5	4	3	2	1	1
.05	5	7	3	2	1	0	0	7	5	4	3	2	1
.05	6	9	5	2	1	0	0	9	8	6	5	3	2
.10	4	14	8	4	1	0	0	14	11	9	7	5	4
.10	5	22	14	8	3	1	0	22	18	15	13	10	7
.10	6	32	22	13	6	2	0	32	28	24	20	16	12
.15	4	30	20	12	6	2	0	30	26	22	18	15	11
.15	5	46	34	22	12	5	1	46	41	36	31	26	21
.15	6	65	49	34	21	10	3	65	59	53	46	40	33

[1] Groups of bulls to select from are:
YB : young bulls with pedigree information
SB : bulls with index based on sib testing
PB : progeny tested bulls

[2] Annual genetic progress in the nucleus herd in phenotypic standard deviations

[3] Years between groups of YB and PB; time between YB and SB assumed to be constant (2 years)

Figure 1: Schematic description of the GxE problem



r_{tc} = GxE correlation test herd -- average commercial herd

r_{cc} = GxE correlation among different commercial herds

Figure 2: Proportion of selected sires which are young bulls (YB, bottom of chart to lower solid (dotted) line), sib-tested bulls (SB, between solid (dotted) lines) and progeny tested bulls (PB, upper solid (dotted) line to top of chart) for different annual genetic trends (10% selection rate) (solid line for $r_{cc}=r_{tc}$)

A) Annual genetic trend .05 sd

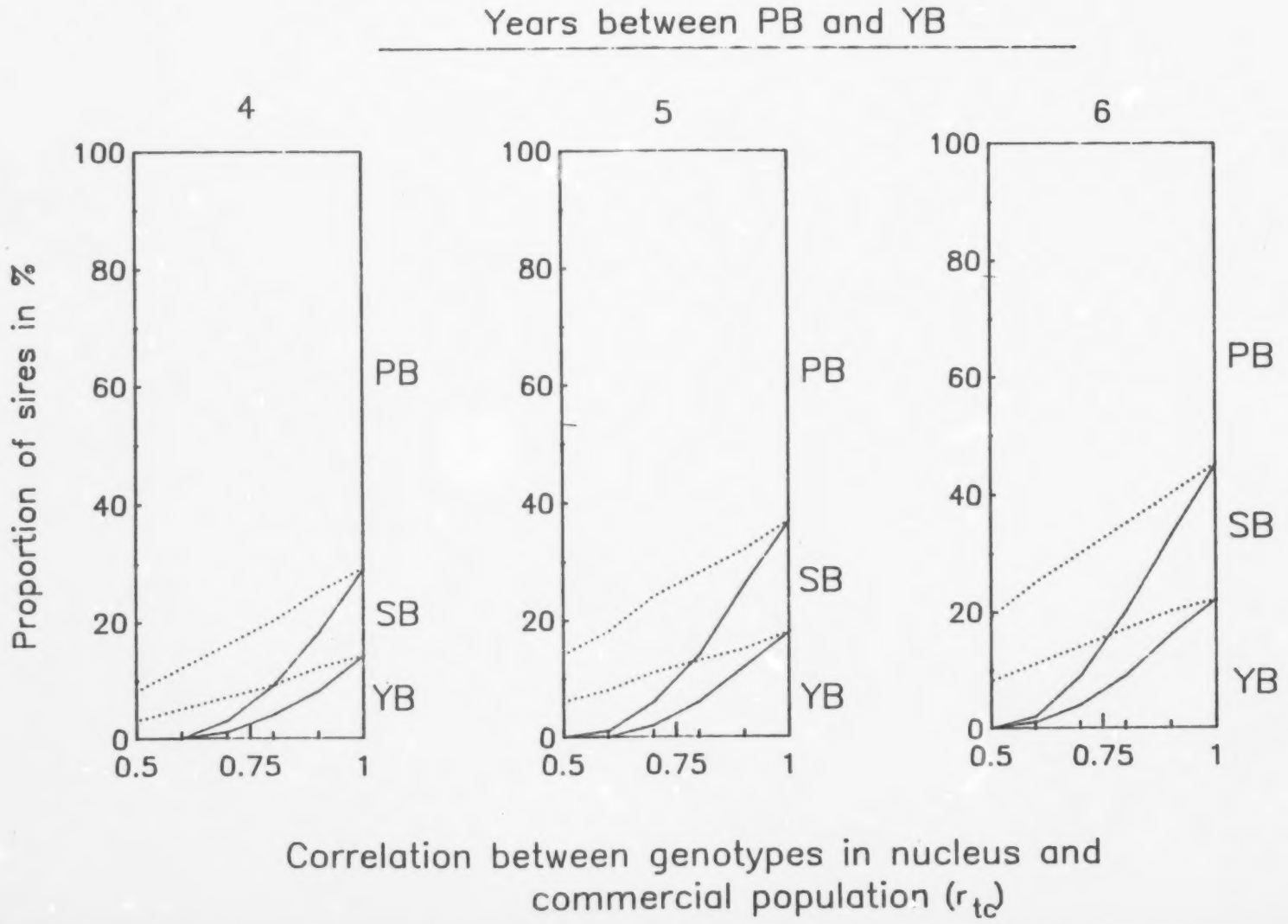


Figure 2: Proportion of selected sires which are young bulls (YB, bottom of chart to lower solid (dotted) line), sib-tested bulls (SB, between solid (dotted) lines) and progeny tested bulls (PB, upper solid (dotted) line to top of chart) for different annual genetic trends (10% selection rate) (solid line for $r_{cc}=r_{tc}$)

B) Annual genetic trend .10 sd

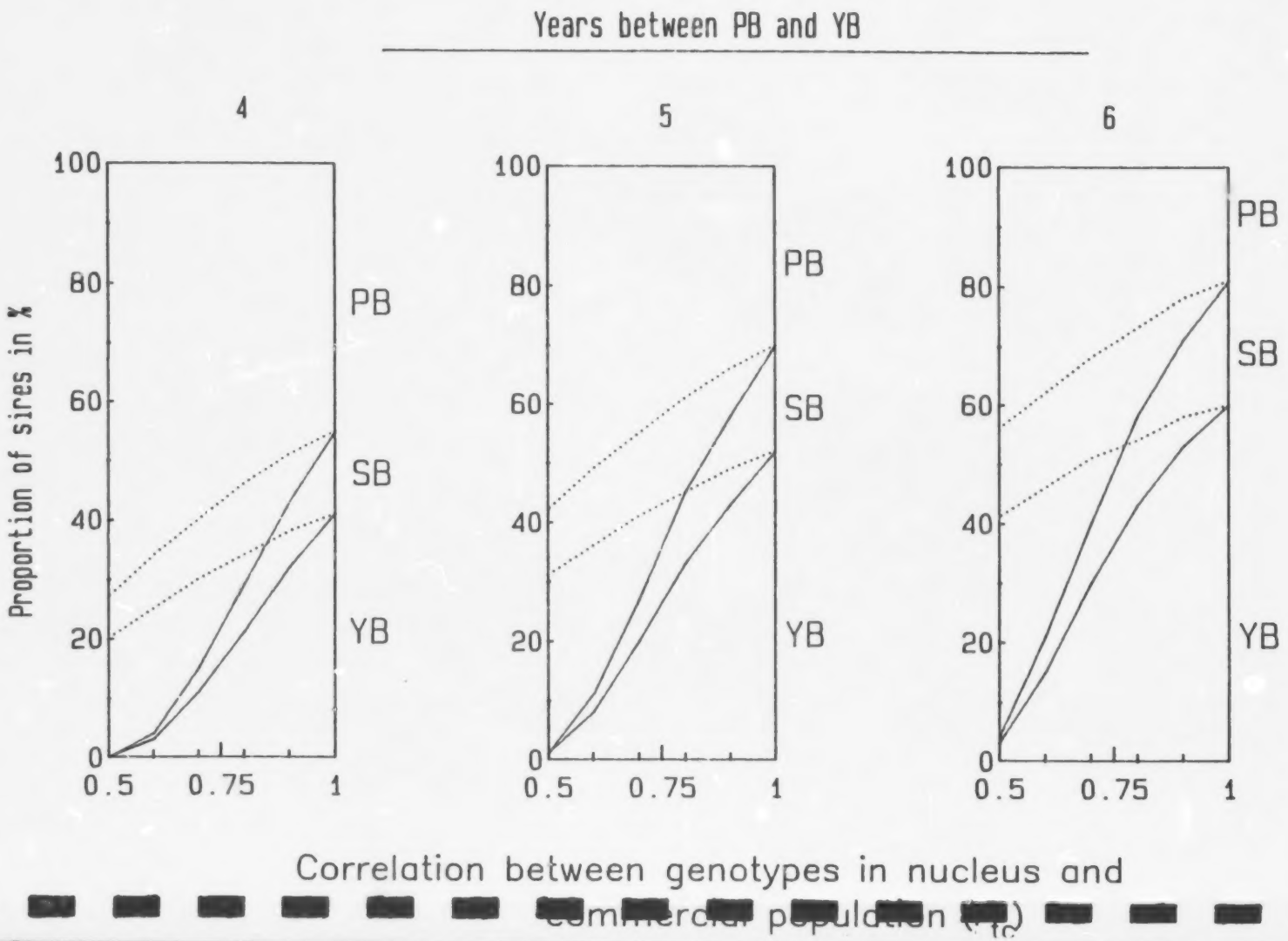
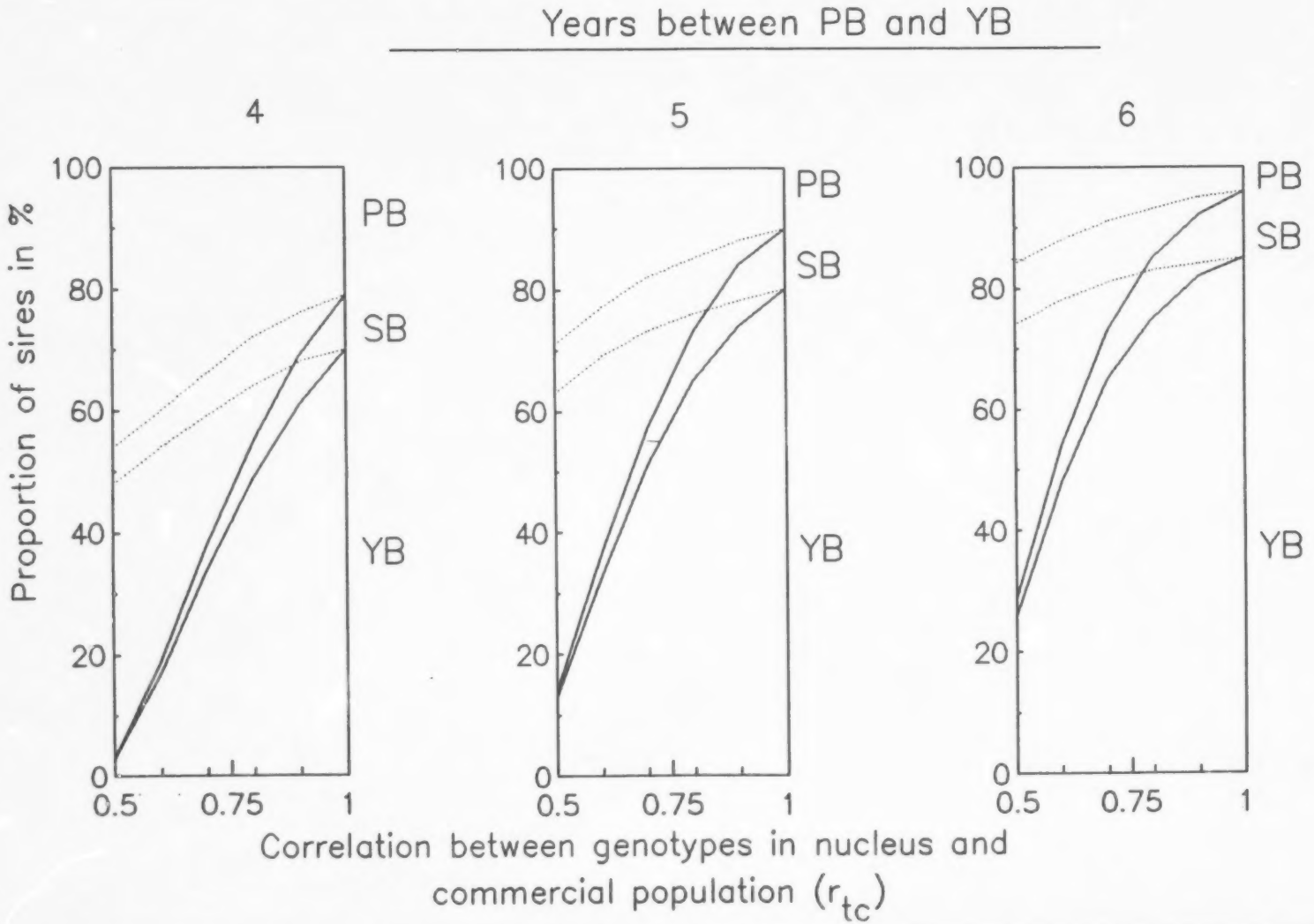


Figure 2: Proportion of selected sires which are young bulls (YB, bottom of chart to lower solid (dotted) line), sib-tested bulls (SB, between solid (dotted) lines) and progeny tested bulls (PB, upper solid (dotted) line to top of chart) for different annual genetic trends (10% selection rate) (solid line for $r_{cc}=r_{tc}$)

C) Annual genetic trend .15 sd



ADDITIVE AND DOMINANCE GENETIC VARIATION FOR PRODUCTION AND CONFORMATION TRAITS IN CANADIAN HOLSTEINS

R.J. Tempelman and E.B. Burnside
Centre for Genetic Improvement of Livestock, Department of Animal and Poultry Science, University of Guelph

SUMMARY

Additive and dominance genetic variances were estimated using restricted maximum likelihood algorithms for two production and nine conformation traits in the Canadian Holstein population. Data for both categories of traits and containing within herd full sisters plus their contemporary herdmates, were analyzed under hierarchical dams within sire models. Heritabilities ranged from 0.32 to 0.41 for production and from 0.10 to 0.30 for conformation traits. Dominance genetic variation as a proportion of the total variation (d^2) was significant for fat yield (0.24), final score (0.15), capacity (0.16) and mammary system (0.13). Both genetic components for milk and fat yield were also estimated under an animal model in four different data sets from three different regions (Western Canada, Eastern Canada and two random data sets from Ontario). Heritabilities ranged from 0.34 to 0.49 for both production traits whereas d^2 was much more heterogenous, ranging from 0.08 to 0.31 for milk yield and 0.08 to 0.50 for fat yield. Dominance genetic variation for fat yield was significant in all regions except Western Canada. Both hierarchical and animal models suggest that genetic variation for milk yield is purely additive.

INTRODUCTION

Dairy cattle breeding programmes have inherently relied upon the existing reproductive technology in effecting genetic progress for economically important traits. Artificial insemination of frozen semen has been the foundation of successful sire progeny testing programmes. Embryo transfer is the latest reproductive phenomenon increasingly used by breeders of Canadian Holsteins. Consequently, the general population structure may be changing from a distribution of paternal half sister families to a more complex split family full sister structure. This would allow dominance genetic relationships to become particularly more prevalent relative to additive genetic relationships. Additive and dominance genetic relationships exist when two animals share relatives on both sides of the pedigree (for example, full sisters) whereas the standard additive genetic relationship may occur when two animals share only one relative. If dominance genetic variance actually exists for economically important traits, the increasing number of dominance genetic relationships could allow for a greater exploitation of total genetic merit (i.e. additive and dominance genetic effects). Dominance genetic effects are defined as intra-locus interaction of gene effects and are generally used to explain hybrid vigour in crossbreeding programmes and depression in animal performance as a consequence of inbreeding. This type of gene action, if significant, may be particularly relevant for corrective mating as well as for multiple ovulation and embryo transfer (MOET) programmes currently in progress.

The objectives of this study were: (1) to partition additive and dominance genetic variances with hierarchical dam within sire models for production and conformation traits and (2) to estimate additive and dominance genetic variances for production traits in regional segments of the Canadian Holstein population incorporating extensive pedigree information in an animal model analysis.

METHODS

First lactation records from 1980-1987 on milk yield and fat yield were obtained from Agriculture Canada. These records were expressed as Breed Class Averages (BCA's) which are production measures adjusted for age and season of calving. First lactation type records from Rounds 37-43 (Years 1983-1987) on final score and the eight major scorecard traits were obtained from the CGIL database. Only within-herd full sister families and their herdmates were included in the analysis. Twin combinations were excluded from the analysis as their dominance genetic relationships were uncertain, i.e. could be 0.25 (dizygotic) or 1.00 (monozygotic), and their similarities may have been augmented by special environmental

effects, e.g. born in same dam parity. In order to account adequately for environmental differences, contemporary subclasses, defined as herd year seasons for production and herd round classifiers for conformation, were required to consist of at least records from five different animals.

Two models were used in the analysis. The hierarchical dams nested within sire model considers only the direct sire and dam relationships of each animal (i.e. does not consider cousin relationships) but does allow a simultaneous analysis of fairly large data sets. A total of 60,892 production records and 65,221 conformation records were analyzed under this model, both of which contained greater than 4500 within herd full sister families. The models for both categories of traits included the fixed effects of contemporary subclasses and genetic group of sires and well as the random effects of sires and dams within sires. Inbreeding coefficients of and relationships among sires due to known male ancestors were also considered. Additional fixed effects fitted for conformation included age at first calving and stage of lactation. The algorithm used for estimating variance components was the derivative-free restricted maximum likelihood (REML) procedure for two random factors. Heritability (h^2) was estimated as four times the sire component divided by the total variance. Dominance genetic variation as a proportion of the total variance (d^2) was estimated as four times the difference between the sire component and the dam within sire component.

The production traits were further studied under an animal model REML variance component procedure. This model made more efficient use of the data as it incorporated all known relationships back to the two grandsires of each animal. All known additive and dominance genetic relationships within herds and all additive relationships due to male ancestors across herd were considered. The model included herd year seasons as fixed effects and an additive and dominance genetic effect as random effects for each animal. Estimating heritabilities and dominance under animal models is much more computationally demanding than computing animal model evaluations. Therefore, only large herds (>50 animals) having full sister families were considered. This left a total of 24,965 records which was still too large for an animal model analysis. Therefore four subsets were created: Western Canada (8239 records), Eastern Canada (5554 records) and 2 random data sets of different herds from Ontario (5733 and 5439 records). Generally, there were 250-400 full sister families along with their contemporary herdmates represented in each subset.

RESULTS AND DISCUSSION

Results computed from the hierarchical model for both categories of traits are presented in Table 1. Heritabilities of all traits were very similar to those previously computed for the Canadian Holstein population. Genetic variation for milk yield appears to be completely additive whereas dominance genetic variance for fat yield significantly accounted for almost 25% of the total variation and 75% of the additive genetic variation. For type traits, dominance genetic variation seemed to be particularly important for final score, capacity, and mammary system and least consequential for dairy character, a trait often estimated to be highly genetically correlated with milk yield. Final score and mammary system would also be expected to have similar results due to previously high sire and error correlations computed between the two traits. It has been hypothesized that dominance is important for broadly defined traits such as final score.

Results for analysis of milk and fat yield under the animal model are presented in Table 2. Heritabilities for both traits were in some cases similar to the estimates from the hierarchical model; however, heritabilities in different regions appeared to be significantly different from each other as approximate standard errors ranged from 0.04 to 0.06. Higher heritabilities are often estimated under an animal model; this was also true for studies involving large New York herds. This observation may be partly due to the incorporation of more relationships, thereby enabling more accurate separation of additive genetic differences from the environment. The Western Canada data set, which had the highest raw means for milk and fat yield, also had the greatest heritability estimates for both traits. This relationship between production level and heritability has been found in other

studies. Conversely, dominance genetic variation for both traits was inconsequential in Western Canadian data whereas dominance genetic variation was significant for fat yield in Eastern Canada and Ontario. Possibly, superior levels of management allow greater expression of additive genetic effects and less expression of dominance genetic effects. Differences in estimates between regions, however, could also be attributed to differences in relationship structures among the four data sets. As previously concluded from the hierarchical model, genetic variation for milk yield would appear to be entirely additive as dominance approached significance for only one data set in the animal model analysis.

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Table 1. Estimates of heritability (h^2) and dominance (d^2) from the hierarchical analysis.

	Production		Conformation								
	Milk yield	Fat yield	FS	GA	DC	CAP	RUMP	F&L	MAMM	FU	RU
h^2	.40	.32	.15	.14	.21	.29	.22	.10	.14	.13	.13
d^2	.06	.24**	.15+	.12	.03	.16*	.07	.06	.13+	.11	.08

** Very significant *Significant +Approaching significance

(Note: All h^2 were very significant)

(FS=final score; GA=general appearance; DC=dairy character; CAP=capacity; RUMP=rump; F&L=feet and legs; MAMM=mammary system; FU=fore udder; RU=rear udder).

Table 2. Estimates of heritability and dominance from animal model analyses of four Canadian subsets of data.

Trait		Eastern Canada	Ontario Subset 1	Ontario Subset 2	Western Canada
Milk yield:					
	h^2	.35	.38	.34	.49
	d^2	.22	.14	.31+	.08
Fat yield:					
	h^2	.34	.47	.41	.48
	d^2	.40*	.38*	.49**	.08

** Very significant *Significant +Approaching significance

(Note: All h^2 were very significant)

THE INVERSE OF THE GAMETIC RELATIONSHIP MATRIX

L. R. Schaeffer and B. W. Kennedy
Centre for Genetic Improvement of Livestock
Animal & Poultry Science
University of Guelph

INTRODUCTION

The gametic relationship matrix was developed to compute the dominance genetic relationship among individuals that might possibly be inbred. Dominance genetic effects may be important in animal evaluation, for example, as an increasing number of full-sib families in dairy cattle result from the increased use of embryo transfers. The elements of a gametic relationship matrix are the probabilities of genes being identical by descent.

CONSTRUCTION OF GAMETIC RELATIONSHIPS

In a diploid species, each animal has two genomes. For individual A let A_1 and A_2 represent the two genomes, and likewise for animals B, C, and D. Suppose that A and B are unrelated individuals, and that C is an offspring of A and B, and D is an offspring of A and C. The gametic relationships for these four individuals and their eight genomes is given in Table 1. Note that all diagonal elements are always equal to one. Let X_iY_j represent the row and column identification for an element in this table, then for example,

$$A_1C_1 = .5 [A_1A_1 + A_1A_2] = .5 [1 + 0] = .5,$$

and

$$A_1D_2 = .5 [A_1C_1 + A_1C_2] = .5 [.5 + 0] = .25.$$

Thus, the recursive nature of the elements is similar to that of the elements of the additive relationship matrix, **A**.

From the gametic relationship table it is possible to calculate both additive and dominance genetic relationships among individuals. The additive genetic relationship, for example, between individuals A and D is

$$\underline{a}_{AD} = .5 [A_1D_1 + A_1D_2 + A_2D_1 + A_2D_2]$$
$$= .5 [.5 + .25 + .5 + .25] = .75.$$

Similarly, the dominance genetic relationship between A and D is

$$\underline{d}_{AD} = [(A_1D_1)(A_2D_2) + (A_1D_2)(A_2D_1)]$$
$$= [(.5) (.25) + (.5) (.25)] = .25.$$

Note that

$$\underline{a}_{DD} = .5 [1 + .25 + .25 + 1] = 1.25,$$

and

$$d_{DD} = [\,(1)\,(1) + (.25)(.25)\,] = 1.0625.$$

THE INVERSE OF THE GAMETIC RELATIONSHIP MATRIX

Research into the application of animal models to the genetic evaluation of dairy cattle has been greatly facilitated by Henderson's discovery of a rapid method for inverting the additive genetic relationship matrix when animals are non inbred. Dr. Quaas(Cornell) extended the procedure to the case of inbred animals, with the requirement that animals had to be ordered chronologically. Using the approach of Quaas, the inverse of the gametic relationship matrix was derived as follows. Let

$$G = LL' = TDDT' \qquad [3]$$

where L and T are lower triangular matrices and D is diagonal. Then

$$G^{-1} = T'^{-1}D^{-2}T^{-1}. \qquad [4]$$

In a gametic relationship matrix, the elements of T^{-1} are all ones on the diagonals and -.5 for each possible 'parent' gamete of the i-th gamete in the i-th row, of which there are always two 'parent' gametes. The diagonal elements of D^{-1} are obtained in a manner similar to Quaas as follows:

Step 1: Order animals and gametes chronologically.

Step 2: Initialize a vector u to null, where u has length equal to the total number of gametes, N.

Step 3: Process each gamete in sequence. For the k-th gamete let

$\theta_k = (1 - u_k)^{.5}$ be the k-th diagonal element of D,

and for j = k to N, let j and m represent the parent gametes of the i-th gamete, (e.g. if i = C_1 in Table 1, then j = A_1 and m = A_2 since C_1 represents the genome from parent A), then calculate

$\lambda_{kk} = \alpha_k$ when i = k,

or $\lambda_{ki} = .5\,(\lambda_{kj} + \lambda_{km})$ when i > k provided that j > k and m > k,

or $\lambda_{ki} = .5\,\lambda_{kj}$ if m < k and j > k,

or $\lambda_{ki} = .5\,\lambda_{km}$ if j < k and m > k,

or $\lambda_{ki} = 0$ if j < k and m < k.

Having determined λ_{ki} then add the square of this number to

u_i. (i.e. $u_i = u_i + \lambda^2_{ki}$)

Step 4: Repeat step 3 for each gamete until all gametes have been processed.

For the example individuals in Table 1, the diagonals of D were found to be

D = diag{ 1 1 1 1 .70711 .70711 .70711 .70711 }.

The elements of the inverse of G can be written directly as follows:

1) For the k-th gamete with parent gametes j and m, then let

$$\underline{c} = 1/(d_k d_k)$$

and accumulate into the inverse matrix the following constants.

Position in inverse	constant to add
(k,k)	$\underline{c}$
(k,j) (j,k) (k,m) (m,k)	$-\underline{c}/2$
(j,j) (m,m) (j,m) (m,j)	$\underline{c}/4$

2) If the parent gametes are unknown then add $\underline{c}$ to the (k,k) position of the inverse.

Note that either both parent gametes are known or both are unknown. The situation of one known and the other unknown does not occur in a gametic relationship matrix.

Using the above rules, the inverse of the gametic relationship matrix in Table 1 is given in Table 2. Thus, the inverse of a gametic relationship matrix is no more difficult than for the inverse of the additive genetic relationship matrix with inbred animals. The main difference is that the order of **G** is twice the order of **A** for diploid species. The gametic relationship matrix could also be constructed for haploid, triploid, or tetraploid species of animals or plants, if necessary. The inverse of **G** under those situations may be more complex to compute.

Table 1. Example gametic relationship matrix.

		A		B		C		D	
		A_1	A_2	B_1	B_2	C_1	C_2	D_1	D_2
A	A_1	1	0	0	0	.5	0	.5	.25
	A_2	0	1	0	0	.5	0	.5	.25
B	B_1	0	0	1	0	0	.5	0	.25
	B_2	0	0	0	1	0	.5	0	.25
C	C_1	.5	.5	0	0	1	0	.5	.5
	C_2	0	0	.5	.5	0	1	0	.5
D	D_1	.5	.5	0	0	.5	0	1	.25
	D_2	.25	.25	.25	.25	.5	.5	.25	1

Table 2. Inverse of the gametic relationship matrix in Table 1.

		A		B		C		D	
		A_1	A_2	B_1	B_2	C_1	C_2	D_1	D_2
A	A_1	2	1	0	0	-1	0	-1	0
	A_2	1	2	0	0	-1	0	-1	0
B	B_1	0	0	1.5	.5	0	-1	0	0
	B_2	0	0	.5	1.5	0	-1	0	0
C	C_1	-1	-1	0	0	2.5	.5	0	-1
	C_2	0	0	-1	-1	.5	2.5	0	-1
D	D_1	-1	-1	0	0	0	0	2	0
	D_2	0	0	0	0	-1	-1	0	2

ESTIMATION OF ADDITIVE MATERNAL AND CYTOPLASMIC GENETIC VARIANCES UNDER ANIMAL MODELS

O.I. Southwood, B.W. Kennedy, K. Meyer[1] and J.P. Gibson
Centre for Genetic Improvement of Livestock, Department of Animal & Poultry Science, University of Guelph, [1]Institute of Animal Genetics, University of Edinburgh

SUMMARY

Two pathways for maternal genetic effects to act on production traits are additive maternal and cytoplasmic. Estimation of these variances have generally assumed that the correct maternal pathway is known. A method is described which allows simultaneous estimation of additive maternal and cytoplasmic genetic variances, as well as additive direct genetic variances and error variances using an animal model. Data were simulated assuming an additive maternal model, cytoplasmic model, or both. The method was tested by analyzing the data under the true or incorrect maternal model using a restricted maximum likelihood procedure, with an animal model which accounted for additive genetic relationships. Additive direct, additive maternal, cytoplasmic and error variances were correctly partitioned when estimated under the true model. However, when additive maternal or cytoplasmic genetic effects were ignored in the model used for analysis, estimates of additive direct variance were significantly inflated. Also, under an additive maternal genetic model, a small cytoplasmic variance was estimated though none was present in the data.

INTRODUCTION

Maternal genetic effects are defined here as genetic effects of a dam additional to her direct additive genetic contribution. They may arise through several pathways, though only the two most usually described will be examined. These are additive maternal genetic and cytoplasmic effects. Additive maternal effects are assumed to be inherited in a Mendelian fashion. Cytoplasmic genes are transferred directly from dam to offspring, and assuming no mutation or heterogeneity of the cytoplasmic components within a dam, all offspring of a dam will receive identical copies. Analyses of maternal effects have generally assumed one or the other of these pathways is the true model but not both.

Usually there is some confounding in the data between direct additive genetic effects and maternal effects. For example, if additive genetic covariances among individuals are not accounted for by the model and procedures used for analysis, spurious estimates of cytoplasmic effects can result but analysis under an animal model can effectively separate direct additive genetic from cytoplasmic effects.

A method is proposed here which allows simultaneous estimation of additive direct and additive maternal genetic, cytoplasmic and error variances under an animal model using derivative-free restricted maximum likelihood (DFREML). The method was tested by analysis of data simulated under either an additive maternal genetic or a cytoplasmic model or both. Bias from analysing data under correct and incorrect genetic models were examined.

PROCEDURES

Data spanning 60 years for a hypothetical trait were generated by computer simulation. Cows were distributed over five herds. Each cow had a single record, and selection and mating were at random.

Simulation was according to one of the following genetic models: additive maternal, cytoplasmic, and maternal+cytoplasmic. The full model (maternal+cytoplasmic) was:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Pm} + \mathbf{Wc} + \mathbf{e}$$

where $\mathbf{y}$ is the vector of observations, $\mathbf{b}$ is a vector of fixed effects, $\mathbf{a} \sim (0, \mathbf{A}\sigma^2_a)$ is a vector of additive direct genetic values, $\mathbf{m} \sim (0, \mathbf{A}\sigma^2_m)$ is a vector of additive maternal genetic values, $\mathbf{c} \sim (0, \mathbf{I}\sigma^2_c)$ is a vector of cytoplasmic effects and $\mathbf{e} \sim (0, \mathbf{I}\sigma^2_e)$ is the vector of residual environmental effects. Also, $\mathbf{A}$ is the matrix of additive genetic relationships among animals and $\mathbf{X}$, $\mathbf{Z}$, $\mathbf{P}$ and $\mathbf{W}$ are incidence matrices and assign the appropriate effects to the vector of production records, and

$$Var(y) = ZAZ'\sigma^2_a + PAP'\sigma^2_m +$$

$$ZAP'\sigma_{am} + PAZ'\sigma_{am} + WW'\sigma^2_c + I\sigma^2_e$$

where σ_{am} is the covariance between additive direct and additive maternal effects. Population parameters were $\sigma^2_a = \sigma^2_m = \sigma^2_c = .3$ and $\sigma^2_e = .7$. Two separate simulations were run. In the first, $\sigma_{am} = 0$ and in the second $\sigma_{am} = .15$. The additive maternal model was according to the full model except cytoplasmic effects (Wc) were deleted and not simulated. Similarly the cytoplasmic model was according to the full model except additive maternal effects (Pm) were deleted. The simulation was replicated ten times for each genetic model.

Simulation of direct additive genetic effects was according to Mendelian principles. The maternal genetic effect was divided into two independent components, additive maternal and cytoplasmic. An offspring received half her additive maternal genes from her sire and half from her dam in a Mendelian manner, but

received the cytoplasmic component only from her dam. The cytoplasmic component was constant from generation to generation. Each foundation female in a maternal line was identified as a cytoplasmic source and cytoplasmic values for founder cows were simulated using a pseudo random number generator. A cytoplasmic line was defined as all animals sharing a common cytoplasmic source. There was an average of 99 cytoplasmic lines per replicate. Additive direct effects were independent of cytoplasmic effects. In some simulations there was a genetic covariance between additive direct and additive maternal effects. No maternal environmental variance was simulated to help simplify comparisons between models.

First lactation records over the final 30 years were analyzed giving a total of 4,500 records. Variance components were estimated under both correct and incorrect models. Data simulated under the maternal+cytoplasmic model were analyzed according to the correct model [1] and incorrect models with additive maternal or cytoplasmic effect ignored. Data simulated under the additive maternal model were analyzed according to an additive maternal model (correct) and cytoplasmic model (incorrect). Similarly, data simulated under a cytoplasmic model were analyzed using a cytoplasmic model (correct) and an additive maternal (incorrect) model.

Variance components were estimated by derivative free restricted maximum likelihood under an individual animal model. The standard deviation among replicates was computed to estimate the sampling variance.

RESULTS

The mean of the sample values for the variance components and heritabilities for the maternal, cytoplasmic and maternal+cytoplasmic data sets are shown in Table 1 (σ_{am}=0) and Table 2 (σ_{am}=.15). Also shown are means of the variances estimated under the three models and standard errors of the means.

When the variances were estimated under the correct model for the data set, estimates of variances were in good agreement with true values. However, when the data set was analyzed by an alternative incorrect model, additive direct genetic variance was significantly inflated ($P<.01$) for most models. The exception was for a data set generated according to the full model but analyzed under the maternal model. For this model, estimates of additive direct and additive maternal variance depended on the value of their genetic covariance. When the covariance was zero (Table 1), the direct and maternal genetic variances were inflated, significantly ($P<.01$) for the maternal variance, and the error variance was significantly reduced ($P<.05$). When the covariance was equal to .15 (Table 2), all variance component estimates except the residual error were within one standard deviation of the true values.

It may be useful to examine what happens to the additive maternal and cytoplasmic variances when estimated using an incorrect genetic model, in this case, from data generated under the cytoplasmic or maternal models respectively. The results were very similar for both covariance values. When data simulated under a maternal model were analyzed by a cytoplasmic model, estimates of cytoplasmic variance were more than one

standard deviation from the true value. With data simulated under a cytoplasmic model but analyzed using a maternal model, estimates of the additive maternal genetic variance were smaller and less than one standard deviation from the true value. These results, however, should be taken with some caution, as the maternal and cytoplasmic variances from these models are subject to a truncated sampling distribution. A likelihood ratio test may be a more appropriate test of significance in these situations.

DISCUSSION

Cytoplasmic effects have been reported to have a significant effect on such traits as milk production in dairy cattle, preweaning traits in beef cattle and carcass traits in swine. However, alternative explanations for these observations were rarely examined. Selection and drift, if not accounted for in the maternal lineage, could produce results interpreted as a cytoplasmic effect even though none may be present.

In the present simulations, small cytoplasmic variances were observed when the data were simulated assuming the presence of additive maternal but not cytoplasmic genetic effects. Such apparent cytoplasmic effects could have arisen from residual maternal effects not partitioned into the additive direct component. Ratios of σ^2_c to σ^2_e observed in this study (.018 to .031) were within the range of other reported estimates from analyses of actual data sets. Therefore, unless the underlying genetic model is understood, it is not known whether reported values are true estimates of cytoplasmic variance or due to random fluctuations of other maternal genetic effects. Although variation within a maternal lineage may exist for mitochondrial DNA, neither this variation, nor the metabolic activity of the mitochondria has yet been linked conclusively with performance traits within a breed.

These results indicated that given the correct genetic model, true values were within the 95% confidence interval of the estimate. It was noted that when variances were estimated following an incorrect model, additive direct variance was inflated significantly. Even under an animal model, if maternal effects are present, but not accounted for correctly in the model, estimated additive direct variance will also include all or part of the maternal variance.

Reanalysis of a data set, including a mitochondrial effect led to an increase in the residual variance and decrease in the additive direct variance. This effect could also be seen with the cytoplasmic data sets in Tables 1 and 2. Also, additive direct variance estimated on data generated under a cytoplasmic model and analyzed under the true model was inflated, though not significantly. It is not clear why this should be so. Possible reasons are that due to the structure of the data within maternal lines, some of the cytoplasmic variance may be partitioned into the additive direct effect. There may also be underlying covariances between additive direct and cytoplasmic effects in the data. Generally, sampling errors were larger when variance components were estimated under an incorrect model, indicating estimated values were more subject to chance variation.

The sampling correlations between variances were also examined. These would give an indication as to the chances of partitioning the variances correctly. None of the correlations between σ^2_a, σ^2_m, σ_{am} or σ^2_c were significantly different from zero, except between σ^2_m and σ_{am} estimated from data generated under a cytoplasmic or maternal+cytoplasmic model and analyzed under a maternal model ($P<.05$). An overestimate of one of the variances resulted in an underestimate of the second in the first data set, while in the second the variance and covariance were positively correlated.

The results demonstrate that use of animal models for variance component estimation, although computationally difficult, can correctly partition variation due to additive direct, additive maternal and cytoplasmic effects, when the correct model is applied to the data. Their use should be encouraged.

ACKNOWLEDGEMENTS

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TABLE 1. Estimates of variance components and heritabilities from data generated under the additive maternal, cytoplasmic or maternal+cytoplasmic (mat+cyt) models ($\sigma_{am}=0$).

True model	Variance component	Mean of sample values	Model used for analysis: Maternal	Cytoplasmic	Mat+cyt
Maternal	σ^2_a	.301	.309(.035)[1]	.534(.043)***	
	σ^2_m	.298	.300(.011)	-	
	σ^2_c	.000	-	.014(.012)	
	σ^2_e	.708	.719(.025)	.793(.034)*	
	h^2_a	.230	.232(.024)	.398(.026)***	
	h^2_m	.228	.226(.008)	-	
	h^2_c	-	-	.011(.009)	
Cytoplasmic	σ^2_a	.301	.733(.094)**	.396(.056)	
	σ^2_m	.000	.010(.015)	-	
	σ^2_c	.292	-	.265(.054)	
	σ^2_e	.708	.572(.037)**	.686(.020)	
	h^2_a	.232	.556(.048)***	.293(.032)	
	h^2_m	-	.008(.012)	-	
	h^2_c	.223	-	.196(.033)	
Mat+cyt	σ^2_a	.301	.401(.047)	.617(.070)**	.332(.036)
	σ^2_m	.298	.524(.057)**	-	.346(.035)
	σ^2_c	.292	-	.287(.056)	.252(.053)
	σ^2_e	.708	.641(.024)*	.766(.033)	.700(.020)
	h^2_a	.188	.256(.026)*	.369(.031)***	.203(.020)
	h^2_m	.187	.334(.024)***	-	.212(.018)
	h^2_c	.181	-	.171(.028)	.154(.028)

[1] Standard error of estimate.

* P<0.05; ** P<0.01; *** P<0.001.

TABLE 2. Estimates of variance components and heritabilities from data generated under the additive maternal, cytoplasmic or maternal+cytoplasmic (mat+cyt) models (σ_{am}=.15).

True model	Variance component	Mean of sample values	Model used for analysis		
			Maternal	Cytoplasmic	Mat+cyt
Maternal	σ^2_a	.297	.288(.044)[1]	.800(.046)***	
	σ^2_m	.302	.309(.058)	-	
	σ_{am}	.150	.139(.031)	-	
	σ^2_c	.000	-	.020(.017)	
	σ^2_e	.703	.690(.035)	.647(.035)	
	h^2_a	.205	.202(.029)	.546(.028)***	
	h^2_m	.208	.216(.029)	-	
	h^2_c	-	-	.013(.011)	
	σ_{am}	.501	.483(.158)	-	
Cytoplasmic	σ^2_a	.297	.729(.061)***	.369(.044)	
	σ^2_m	.000	.000(.000)	-	
	σ_{am}	.000	.000(.000)	-	
	σ^2_c	.271	-	.254(.041)	
	σ^2_e	.703	.554(.036)**	.675(.021)	
	h^2_a	.234	.567(.035)***	.284(.029)	
	h^2_m	-	.000(.000)	-	
	h^2_c	.212	-	.195(.027)	
	σ_{am}	-	.109(.244)	-	
Mat+cyt	σ^2_a	.297	.294(.029)	.853(.069)***	.301(.042)
	σ^2_m	.302	.326(.057)	-	.321(.052)
	σ_{am}	.150	.169(.041)	-	.158(.024)
	σ^2_c	.271	-	.292(.058)	.258(.048)
	σ^2_e	.703	.759(.058)*	.634(.034)	.684(.028)
	h^2_a	.173	.190(.016)*	.480(.035)***	.175(.024)
	h^2_m	.175	.210(.029)***	-	.186(.027)
	h^2_c	.157	-	.164(.030)	.150(.026)
	σ_{am}	.501	.542(.082)	-	.521(.126)

[1] Standard error of estimate.

* P<0.05; ** P<0.01; *** P<0.001.

ANTIBODY RESPONSES TO HUMAN ERYTHROCYTES AND OVALBUMIN AS MARKER TRAITS OF DISEASE RESISTANCE IN DAIRY CALVES

Jeanne L. Burton, E.B. Burnside, B.W. Kennedy, B.N. Wilkie[1] and J.H. Burton
Centre for Genetic Improvement of Livestock, Department of Animal & Poultry Science, University of Guelph and [1]Department of Veterinary Microbiology and Immunology, University of Guelph

SUMMARY

Microhemagglutination procedures were used to quantify antibody titers raised against human erythrocytes and ovalbumin in calf sera. Calves were also monitored for the prevalence of pneumonia and diarrhea. Calves, 72 males and 82 females, were the progeny of 15 artificial insemination bulls. Blood was sampled weekly for 2 wk post primary and secondary immunizations. Antibody response peaks to both antigens occurred by 14 and 7 d post immunization, respectively. There were some significant effects of season of birth and location of rearing on antibody titers against both test antigens. Diarrhea prevalence was negatively associated with high primary response antibody titers against human erythrocytes, but no trends were observed for pneumonia prevalence or for antibody titers to ovalbumin. Paternal half-sib heritability estimates ranged from 0 to .40$\pm$.32 for primary antibody responses and from 0 to .87$\pm$.50 for secondary antibody responses, depending on antibody specificity and those for average titer were higher for anti-ovalbumin antibody (h^2 = .48$\pm$.39) than for anti-human erythrocyte antibody (h^2 = .31$\pm$.31). Heritabilities of this magnitude suggest the feasibility for successful genetic manipulation of antibody response profiles of young calves, and these may contribute to enhanced disease resistance.

INTRODUCTION

Heritability estimates of diseases in calves and cows are generally low, about .05 or less. Well defined immunological marker traits, presumably indicative of disease resistance capacity, may have higher and more accurate heritability estimates than health traits and may provide an efficient indirect means of genetically altering disease resistance in domestic animals. This would be of particular importance where pathogen eradication and/or disease control is not possible by more economical means (eg. environmental cleanliness and/or vaccination).

Feasibility of marker assisted selection for disease resistance is based on finding reliable immunological markers which are easily measured, preferably in young animals of both sexes, and are indicative of resistance to economically important diseases. Practical application would be through a challenge test using harmless test antigens which fit all of the required criteria of a marker trait for genetic improvement of disease resistance.

The objectives of this study were to examine the serum antibody responses to two test antigens, human erythrocytes (HRBC) and ovalbumin (OVA), in 3 wk old colostrum fed dairy calves to determine sources of variation in these responses, to relate the humoral responses to prevalence and severity of naturally occurring neonatal diarrhea and pneumonia, and to estimate paternal half-sib heritabilities of primary and secondary humoral responses.

PROCEDURES

Design

A total of 154 (72 male and 82 female) calves, sired by 15 artificial insemination bulls, were born over three batch calving seasons between 1983 and 1984, in the University of Guelph - Elora Dairy Research herd. At birth, calves were removed from their dams prior to suckling and fed 1.75L of dam's colostrum from a nipple bottle within 6 h. Calves were fed an additional 1.2 kg pooled colostrum at the subsequent milking, within approximately 12 h of the first colostrum feeding. At day 1 of age, calves were moved to one of three rearing locations, where they were fed milk from buckets twice daily at approximately 2.5% of body weight per feeding. Long hay and commercial calf starter were offered ad libitum from day 1 of age, and water was given after weaning at 6 wk (day 42) of age.

Venous blood was sampled at weekly intervals starting at day 21 (0 d post immunization) and continuing on days 28 and 35 of age (7 and 14 d post primary immunization) and days 42 and 49 of age (7 and 14 d post secondary immunization). Immunizations with test antigens occurred immediately after blood sampling on day 21 and day 35 of age. The day 21 sample was intended as preimmunization serum, and that from day 35 as the last sample from the primary response.

Test Antigens and Serology

Human erythrocytes (HRBC) and ovalbumin (OVA) were the antigens chosen for immunization of calves since they were complex, foreign and harmless. At each immunization (days 21 and 35 of age), 2 ml of the 20% HRBC suspension were injected via the right jugular vein. At 21 d and 35 d of age, each calf received a total of 4 ml of adjuvanted OVA, subcutaneously and bilaterally in the suprascapular area.

Calf serum antibody to HRBC was titrated by standard direct microhemagglutination procedure using Type A (Rh+) washed packed HRBC suspended to 1% (V/V) in 1% heat inactivated fetal calf serum-PBS as antigen. Serum antibody to OVA was titrated by passive microhemagglutination. Because of its solubility, OVA was conjugated to sheep erythrocytes (SRBC) by means of the agent 1-ethyl-1, 3-(3-dimethylaminoprophyl) carbodiimide hydrochloride (ECDI) to facilitate visualization of the agglutination.

Disease Prevalence

Because of their frequencies in the calf herd, pneumonia and diarrhea were the diseases of interest in this study. If either disease was diagnosed during daily monitoring of calf health, treatment dates were recorded on Research Station calf health cards.

Prevalence of diarrhea or pneumonia was classified as all-or-none, indicating if there was treatment at least once for disease during the 7 wk trial. From these diarrhea and pneumonia scores, a disease categories referred to as "sick" was created indicating freedom from both diseases during the entire trial (0), the calf was treated at least once for one of the two diseases (1), or the calf was treated at least once for both diseases (2).

Severity of diarrhea, pneumonia and sick was extracted from the calf health records and was considered the total number of days of treatment during the trial.

Statistical Analysis

Least squares analyses were performed on $\log_e$ transformed data in five separate steps in an attempt to distinguish the effects of managerial-type variables from disease-type variables. Model I was created to test for fixed managerial effects of season, location, sex, twinning and sire; Model II to test for the effects of pneumonia and diarrhea prevalence adjusted for managerial effects; Model III to test for the effects of severity of pneumonia and diarrhea, adjusted for managerial effects and for the prevalence of each disease; Model IV to test for the effect of sick adjusted for managerial effects, and Model V to test for the effect of sick severity and its prevalence adjusted for managerial effects.

Heritability Estimates

Sire and error variance components were estimated by restricted maximum likelihood (REML) according to Model I, except that the effect of twin was removed. Twins were deleted to remove full-sibs from the data set by randomly discarding one record of each twin set and retaining the other to conserve data. Sire and error variances were used to estimate paternal half-sib heritabilities.

RESULTS

Serum Antibody Profiles

Mean serum antibody profiles on raw and $\log_e$ transformed data are given in Table 1. Although variation in response to HRBC and OVA was very large, on average very young calves were able to respond to these test antigens.

Although preimmunization antibody titers were essentially zero for both test antigens,

their standard deviations (Table 1) suggest that either the serological test gave low false positives for some sera, or that there was a degree of cross-reactivity between test antigens and those that calves were naturally exposed to.

Under the blood sampling protocol, peak primary antibody responses to both HRBC and OVA occurred somewhere between 7 to 14 days post-immunization (Table 1). The response to HRBC by 14 days post primary immunization was 3 times higher than that to OVA. Primary antibody response to OVA was almost twice as variable as the primary response to HRBC.

Contrary to the primary responses, secondary antibody responses to both HRBC and OVA peaked by 7 days post immunization and were relatively invariable. Furthermore, variation was of the same magnitude for both test antigens (Table 1).

Correlations

Preimmunization titers to both HRBC and OVA were not related to primary or secondary responses at any phase, while correlations between titers from all other sampling periods (within each antigen-specific response) were positive. Therefore the individuals demonstrating positive titers to either antigen in preimmunization sera represented false positive serological results rather than cross-reactivity of antigens.

From day 28 of age on, the correlations suggested a residual effect of antibody from one week to the next, as well as from each week on average titer for all weeks. For the secondary response, there was a positive relationship between peak primary antibody response titers (at day 35 of age) and subsequent secondary response titers (at days 42 (peak) and 49 (post peak) of age). Furthermore, calves that responded well in the primary response had significantly higher antibody titers to both test antigens when response was calculated as the average titer over both primary and secondary responses.

In a practical sense, the correlations indicate that a single blood sample, taken at or after peak primary response, is sufficient to indicate the calf's ability to respond to each test antigen. Because these correlations are only moderately high, a single sample would only distinguish high from low responders, as the individual calf's relative ranking in absolute titer would change somewhat with time. However, for the purpose of genetically enhancing general disease resistance in calves, "minimum" culling criteria would likely be met by defining low versus high responders.

There was only a small and inconsistent relationship between HRBC and OVA antibody profiles. There was evidence for a positive relationship of peak primary response to HRBC (at day 35) with peak primary and entire secondary response to OVA (days 35 through 49). In addition, from day 35 of age on, average antibody titers to both test antigens were significantly, albeit lowly, positively correlated.

In general, antibody profiles to primary and secondary immunizations with HRBC and OVA are not highly related. Since HRBC and OVA are unrelated antigens it is feasible that these simulate at least part of the variety of natural antigenic exposure of calves, and responses to them would, therefore, partially mimic responses required by the calf to fight infection and/or disease. It is, therefore, realistic that immune profiles from several unrelated test antigen immunizations be required for a disease resistance index.

Managerial Effects

The least squares means for season of birth, location of rearing, sex of calf and twinning effects on antibody responses to HRBC and OVA are given in Tables 2 and 3, respectively. Season of birth influenced preimmunization, 7 day (pre-peak primary) and peak secondary (d 42 of age) titers to HRBC, fall-born calves having higher titers than spring or winter-born calves. The same relationship was seen for antibody titers raised against OVA and included the titers 14 days after secondary immunization as well as average antibody titer over the entire sampling period.

Location of rearing had significant effects on antibody profiles to HRBC for the

entire primary and secondary immune responses (Table 2). Calves reared in individual pens on slatted floors (coded 1) had higher responses than those reared in individual pens on solid floors (coded 2) or those reared in group pens on solid floors (coded 3).

The sex of calf was not significant in determining high or low antibody profiles to HRBC antigens (Table 2), except for a female advantage for peak secondary response (at day 42 of age). Similarly, sex of calf did not influence the antibody profiles to OVA (Table 3).

The effect of twinning was not significant for any phase of the antibody profile to HRBC (Table 2), but non-twin calves had higher antibody titers to OVA at peak primary (day 35 of age) and for average titer than did calves of twin sets (Table 3).

Disease Effects

Pneumonia prevalence did not significantly effect humoral antibody profiles to HRBC or OVA immunizations (Table 4). Diarrhea occurrence affected primary antibody titers to HRBC, but did not affect any part of the antibody profile to OVA. For the anti-HRBC primary antibody response, calves that did not require treatment for diarrhea at least once during the 7 wk trial had significantly higher anti-HRBC antibody titers than did treated calves.

The effects of severity of pneumonia and diarrhea, sick prevalence and severity of sick were not significant for any phase of the antibody response profiles to HRBC or OVA.

Heritability Estimates

Paternal half-sib heritability estimates for antibody profiles against HRBC and OVA are given in Table 5. Except in the secondary immune response to HRBC, peak titers were more highly heritable than initiation or termination titer, and on the whole, the antibody profile against OVA was more highly heritable than that against HRBC. Since little was known regarding natural antigenic exposure of the few individuals showing slight preimmunization titers to each antigen, and since the correlations suggested that positive preimmunization sera were a result of false positive serological results, the implications regarding heritabilities of preimmunization titers are not clear.

From the heritability estimates, it appears that the calf's genotype influences peak response titers more strongly than initiation titers, at least for the primary antibody response to HRBC, and for both primary and anamnestic responses to OVA. Because antibody responses to HRBC and OVA are moderately to highly heritable, success at creating high and low responder lines through sire selection is anticipated.

CONCLUSIONS

Antibody titers raised against HRBC and OVA in the sera of very young calves peaked at 14 and 7 d post primary and secondary immunizations (respectively). Response profiles to both antigens were associated with substantial phenotypic variation in the primary phase, but with relatively less variation in the secondary phase. Part of the variation was managerial oriented, but was accounted for in the statistical models dealing with disease prevalence and heritability estimation. Correlations suggested that screening of high versus low responder calves be accomplished by a single blood sample taken at or after peak primary titers but that antibody responses to a battery of individual antigens be tested independently. This was further verified by the fact that anti-HRBC primary antibody titers were significantly associated with disease prevalence while those raised against OVA were not, even though heritability estimates were relatively high for both profiles at this phase of response. It is anticipated that improved disease resistance of neonatal calves could be achieved by indirect selection based on marker traits (here, anti-HRBC primary antibody response titers), and that response to selection could be as large as that achieved by direct selection based on disease prevalance traits.

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Table 1. Mean antibody titer and $\log_e$ of antibody titer plus one to human erythrocyte (HRBC) and ovalbumin (OVA) antigens in calf serum.

Antibody specificity	Days post-immunization	Age of calf	n	Titer			$\log_e$ plus one titer		
				Mean	±SD	CV(%)	Mean	±SD	CV(%)
HRBC	0	d21[1]	154	2.6	7.4	283.9	.812	.814	100.3
	7	d28	153	171.7	214.0	124.6	3.918	1.93	49.3
	14	d35[2]	153	187.5	263.1	140.3	4.465	1.437	32.2
	7	d42	154	1398.7	1215.7	86.9	6.753	1.162	17.2
	14	d49	154	291.4	383.9	131.7	5.028	1.174	23.4
	Avg.						4.186	.895	21.4
OVA	0	d21[1]	154	.1	.7	1021.8	.021	.197	922.5
	7	d28	153	13.9	36.5	262.3	1.444	1.432	99.1
	14	d35[2]	153	59.4	127.5	214.8	3.022	1.511	50.0
	7	d42	154	843.7	988.3	117.1	6.081	1.314	21.6
	14	d49	154	769.2	813.4	105.7	6.117	1.132	18.5
	Avg.						3.329	.871	26.2

[1] Primary immunization.

[2] Secondary immunization.

Table 2. Least squares means for effects of season, location, sex and twinning on calf serum antibody responses (log_e of titer plus one) to human erythrocyte (HRBC) antigens.

Independent variable	n	Days Post Immunization 0 / Calf Age d21[1]	7 / d28	14 / d35[2]	7 / d42	14 / d49	Avg.
Season		(P=.015)[3]	(P=.001)	(P=.534)	(P=.045)	(P=.889)	(P=.033)
Spring	58	.37[b]	4.89[a]	4.71	6.13[b]	5.00	4.25
Fall	47	1.07[a]	5.48[a]	4.94	7.02[a]	5.08	4.72
Winter	49	1.13[ab]	2.17[b]	4.19	7.24[ab]	4.79	3.91
Location		(P=.253)	(P=.004)	(P=.029)	(P=.027)	(P=.117)	(P=.007)
1	27	.55	4.64[a]	5.40[a]	7.51[a]	5.50[a]	4.74[a]
2	76	1.05	4.47[a]	4.27[b]	6.40[b]	4.67[ab]	4.19[ab]
3	56	.96	3.43[b]	4.18[b]	6.47[b]	4.70[b]	3.95[b]
Sex		(P=.130)	(P=.287)	(P=.080)	(P=.050)	(P=.119)	(P=.337)
Male	72	1.02	4.41	4.28	6.48[b]	4.70	4.19
Female	82	.69	3.95	4.95	7.11[a]	5.21	4.40
Twin		(P=.330)	(P=.332)	(P=.769)	(P=.663)	(P=.570)	(P=.760)
No	142	.72	3.91	4.69	6.88	5.07	4.25
Yes	12	.99	4.45	4.55	6.71	4.84	4.34

[1] Primary immunization.

[2] Secondary immunization.

[3] Probability associated with type III sums of squares.

[a,b] Means with different superscripts within a variable sub-column differ (P<.05).

Table 3. Least squares means for effects of season, location, sex and twinning on calf serum antibody responses (log_e of titer plus one) to ovalbumin (OVA) antigens.

Independent variable	n	Days Post Immunization: 0 / Calf Age d21[1]	7 / d28	14 / d35[2]	7 / d42	14 / d49	Avg.
Season		(P=.004)[3]	(P=.0001)	(P=.464)	(P=.025)	(P=.053)	(P=.003)
Spring	58	-.07[b]	-.40[b]	2.42	5.20[b]	5.48[b]	2.53[b]
Fall	47	-.05[b]	1.48[a]	2.93	6.21[a]	6.32[a]	3.38[a]
Winter	49	.31[a]	2.71[a]	2.53	5.93[ab]	6.08[ab]	3.51[a]
Location		(P=.533)	(P=.708)	(P=.033)	(P=.436)	(P=.688)	(P=.297)
1	22	.10	1.37	2.27	5.78	6.16	3.14
2	76	.05	1.08	2.46	5.60	5.77	3.00
3	56	.03	1.34	3.15	5.97	5.96	3.29
Sex		(P=.409)	(P=.384)	(P=.177)	(P=.097)	(P=.143)	(P=.085)
Male	72	.04	1.12	2.36	5.51	5.74	2.95
Female	82	.08	1.41	2.89	6.05	6.19	3.33
Twin		(P=.191)	(P=.184)	(P=.015)	(P=.071)	(P=.125)	(P=.028)
No	142	.01	1.55	3.24[a]	6.16	6.26	3.44[a]
Yes	12	.11	.98	2.02[b]	5.40	5.66	2.83[b]

[1] Primary immunization.

[2] Secondary immunization.

[3] Probability associated with type III sums of squares.

[a,b] Means with different superscripts within a variable sub-column differ ($P<.05$).

Table 4. Least squares means for effects of pneumonia and diarrhea prevalence[1] on serum antibody responses (log_e of titer plus one) to human erythrocytes (HRBC) and ovalbumin (OVA) antigens.

Independent variable	n	Days Post Immunization with HRBC: 0 Calf Age d21[2]	7 d28	14 d35[3]	7 d42	14 d49	Avg.
Pneumonia		(P=.110)[4]	(P=.416)	(P=.351)	(P=.070)	(P=.977)	(P=.432)
No	116	.89	3.96	4.35	6.88	5.01	4.23
Yes	38	.57	3.63	4.68	6.33	5.01	4.07
Diarrhea		(P=.914)	(P=.039)	(P=.026)	(P=.692)	(P=.543)	(P=.196)
No	129	.72	4.21[a]	4.90[a]	6.55	4.92	4.28
Yes	25	.74	3.38[b]	4.13[b]	6.67	5.11	4.02

	n	Days Post Immunization with OVA: 0 Calf Age d21[2]	7 d28	14 d35[3]	7 d42	14 d49	Avg.
Pneumonia		(P=.584)[4]	(P=.947)	(P=.619)	(P=.805)	(P=.787)	(P=.988)
No	116	.06	1.33	2.51	5.72	5.88	3.10
Yes	38	.03	1.31	2.69	5.64	5.80	3.10
Diarrhea		(P=.689)	(P=.473)	(P=.365)	(P=.417)	(P=.303)	(P=.510)
No	129	.05	1.21	2.77	5.80	5.99	3.16
Yes	25	.03	1.44	2.44	5.55	5.70	3.03

[1] Disease prevalence was scored as all (yes) or none (no) based on treatment at least once during the 49 day trial.

[2] Primary immunization.

[3] Secondary immunization.

[4] Probability associated with type III sums of squares.

[a,b] Means with different superscripts within a variable sub-column differ (P<.05).

Table 5. Paternal half-sib heritabilities for $\log_e$ of serum antibody response titers plus one to human erythrocytes (HRBC) and ovalbumin (OVA).

Antigen	Response phase	Calf age (days)	Days Post Immunization	Heritability	±SE
HRBC	preimmunization	21[1]	0	.51	.42
	primary	28	7	.13	.18
	primary	35[2]	14[3]	.40	.32
	secondary	42	7[4]	.00	–
	secondary	49	14	.00	–
	average titer	–	–	.25	.31
OVA	preimmunization	21[1]	0	.13	.23
	primary	28	7	.00	–
	primary	35[2]	14[3]	.34	.31
	secondary	42	7[4]	.87	.50
	secondary	49	14	.46	.42
	average titer	–	–	.48	.39

[1]Primary immunization.

[2]Secondary immunization.

[3]Corresponds to peak primary antibody titer under the present blood sampling protocol.

[4]Corresponds to peak secondary antibody titer under the present blood sampling protocol.

DINITROCHLOROBENZENE CONTACT HYPERSENSITIVITY AS A MARKER TRAIT FOR SELECTION TO IMPROVE DISEASE RESISTANCE IN CALVES

Jeanne L. Burton, B.W. Kennedy, E.B. Burnside, B.N. Wilkie[1] and J.H. Burton
Centre for Genetic Improvement of Livestock, Department of Animal and Poultry Science, [1]Department of Veterinary Microbiology and Immunology, University of Guelph

SUMMARY

Measurements of double skin fold thickness (mm) were used to assess the feasibility of use of cutaneous delayed-type hypersensitivity response as a marker trait to select for genetically improved disease resistance. Data were from a sample of 149 7 wk old Holstein calves, from 15 sires, that had been previously sensitized to the contact allergen dinitrochlorobenzene (DNCB). Response was monitored 24 and 48 h post challenge. Analysis was by least squares according to a model that included fixed managerial effects, disease prevalence and severity categories and sire of calf. Double skin fold thickness increased about 50% by 24 h, followed by a 4% decrease between 24 and 48 h. Season of birth was the only consistently significant fixed effect. Fall-born calves showed larger skin changes at each stage of response than Spring-born calves. Restricted maximum likelihood estimates of paternal half-sib heritability were moderate to high for all stages of response, and phenotypic and genetic correlations between stages were significant and positive. However, the prevalence and severity of naturally occurring pneumonia and diarrhea did not significantly affect quantity of response.

INTRODUCTION

There are many management tools capable of contributing to improved disease resistance of dairy cattle (and calves). The most immediately satisfying manipulations will likely result from pathogen eradication in combination with rigorous vaccination programs. However, where these methods are apparently unsuccessful, such as in cases of neonatal bovine diarrhea and pneumonia in calves and mastitis in milking cows, short term disease resistance goals must be changed to longer term solutions. One means of accomplishing this may be through efficient sire selection programs. The choice of selection criteria is not clear, and heritability estimates of disease incidence traits are consistently low (usually 5% or less).

A logical alternative to disease incidence traits is to study indirect marker traits. Immunological profiles may be more heritable than direct measures of disease incidence. There is evidence for substantial genetic variation in humoral immune responses of cattle but genetic variation in cell mediated immune responses (CMI) has not been studied in depth. Intracellular bovine pathogens are dealt with predominantly by active CMI, which includes the generation of specific killer and inflammatory mediating T lymphocytes in previously exposed individuals. Antigen-driven skin tests called delayed type (cutaneous) hypersensitivity (DTH) are convenient indicators of the ability to mount active CMI.

The objectives of this study were: to identify potential sources of variation in the contact DTH response of young dairy calves percutaneously sensitized and challenged with DNCB; to examine relationships between this response and the prevalence of all-or-none scored disease (pneumonia and diarrhea) traits; and to estimate paternal half-sib heritabilities for the 24 h and 48 h post challenge phases of the response. The intent of these objectives was to discover if DNCB-induced contact DTH response could provide a feasible marker trait for selection to improve disease resistance.

PROCEDURES

Experimental Design

Sixty-nine male and 80 female Holstein calves, sired by 15 artificial insemination bulls and born in the University of Guelph-Elora Dairy Research herd were put on trial from birth to 53 d of age. These included all available calves, with a range of 1 to 42 calves per sire. Calves were born in a maternity wing over three defined calving seasons (Spring, Fall and Winter) between 1983 and 1984 and were

subsequently reared in one of three locations (called 1, 2 and 3 for individual pens on slatted floors, individual pens on solid floors and group pens on solid floors, respectively) within the research facility. There was a total of six twin sets born.

All calves were removed from their dams prior to suckling, after which each received 1.75L of dam's first colostrum within 6 h of birth. Calves were fed an additional 1.2 kg of pooled colostrum at the milking subsequent to their birth and within approximately 12 h of the first colostrum feeding. At 1 d of age, calves were weighed (considered birth weight, kg) and moved to an available rearing location. Until 42 d of age calves were bucket fed warm pooled milk twice daily at approximately 2.5% of body weight per feeding, and offered long hay and commercial calf starter ad libitum. Water was offered ad libitum along with feed after 42 d of age.

Disease Prevalence and Severity

Daily health of calves was monitored by three regular calf attendants. If disease was diagnosed, treatment was made and then recorded on Research Station calf health cards. Because of high frequencies relative to other diseases, pneumonia and diarrhea were chosen for this study.

The prevalence of both pneumonia and diarrhea was classified as all-or-none, depending upon whether or not they received treatment at least once during the 53 day trial. Combinations of disease prevalence scores were then made to create a disease variable which was called "sick" and coded as "0" for those calves being free of (i.e. never treated for) both diseases, "1" for those calves treated for one of the two diseases and "2" for those calves treated for both diseases during the 53 day trial. Disease severity was also considered for each of pneumonia, diarrhea and sick, and was scored as the total number of days treated during the 53 day trial for each.

Dinitrochlorobenzene Challenge

On 42 d of age, an area of hair bordered by the coxal tuber, ischiatic tuber and sacral vertebrae was clipped from the calves in a square approximately 15 x 15 (cm). A small portion of the clipped area was used for sensitization to DNCB. This was accomplished by percutaneous application and absorption of 0.2 ml 90% dimethyl sulfoxide (DMSO) and a few moments later by 0.2 ml 5% (w/v) DNCB in absolute alcohol. Sensitization was repeated at the same site and in the same manner at age 44 d.

On 51 d of age, two other sites within the clipped area that were medial and adjacent to the sensitization site and approximately 10 cm apart from each other, were measured for double fold skin thickness using calipers accurate to 0.1 mm. The site furthest from the calf's midline was reserved for control (vehicle) solution and that closest to the midline for DNCB challenge (or test) solution. Prechallenge measurements (mm of skin) at control and test sites were coded C0 and T0, respectively. Immediately subsequent to skin thickness measurement, 0.5 ml vehicle (4:1, acetone: olive oil) was applied to the control site and 0.5 ml 5% (w/v) DNCB in the same vehicle applied to the test site.

Exactly 24 and 48 h post DNCB challenge, on d 52 and 53 of age, double skin fold thicknesses at control and test sites were measured. The 24 and 48 h control and test site measurements were coded respectively C24, C48, T24, T48. The intensity of the cutaneous DTH, as measured by double skin fold thickness at 24 and 48 h post DNCB challenge, was then used as an indicator of ability to mount CMI in calves.

CMI was scored in several different ways in an attempt to examine specific portions of the response. In order to determine DTH response to DNCB without the effects of vehicle and inherent skin thickness, test and control sites were subtracted from each other for 24 h (DTC24) and 48 h (DTC48) measurements. The DTC24 and DTC48 scores, therefore, indicated magnitude of response to treatment. To assess the various phases of cutaneous DTH, in the absence of inherent skin thickness and vehicle effects, test site measurements were subtracted from each other as follows: 24 h minus prechallenge (DT240) to give an indication of rate of onset of response; 48 h minus 24 h (DT4824) to reflect persistency of peak

response; and 48 h minus prechallenge (DT480) to represent duration of total response.

Statistical Analysis

Least squares analyses were performed on five models to assess managerial, disease prevalence and severity effects on cutaneous DTH. Model I was used to test known, fixed managerial effects of season, location, sex, twinning, calving ease and birth weight and the effects of sire. Models II, III, IV and V were used to test effects of disease prevalence and severity after adjusting for the effects in Model I.

Heritability Estimates

Sire and error variance components were estimated by restricted maximum likelihood (REML) according to Model I, except that the effects of birth weight, calving (birth) ease and twin were removed. Birth weight was deleted to avoid the inclusion of a heritable trait potentially related to skin thickness. Calving ease was removed because it was not a significant effect for any measurement of cutaneous DTH, and twins were deleted to remove full-sibs from the data set. One record of each twin set was randomly discarded and the other retained to conserve data. Sire and error variances were then used to estimate paternal half-sib heritabilities for all measurements of cutaneous DTH.

RESULTS AND DISCUSSION

Cutaneous DTH Profiles

Means, standard deviations and coefficients of variation for response of calves to percutaneous application of DNCB are given in Table 1. There was a slight increase in double skin fold thickness at the control site at 24 h ($P>.05$) and 48 h ($P<.05$) after vehicle application, consistent with the minimal swelling from acetone in olive oil. The prechallenge control (CO) and test site (TO) measurements were nearly identical.

Mean responses in double skin fold thickness to DNCB challenge were substantial, with peak response occurring at roughly 24 h post challenge (T24) and a mean decline of 4% of T24 by 48 h of response (T48; Table 1). This was reflected in an average decrease of -.31 mm of skin fold thickness at the test site between 24 and 48 h post challenge (DT4824).

Managerial Effects

Least squares means for effects of season of birth and sex of calf, least squares estimates for birth weight effects are given in Table 2. Location of rearing, twin versus non-twin calves and ease of birth were not related ($P>.10$) to any measurement of cutaneous DTH response to DNCB.

Season of birth influenced DTH response. Fall calves had generally higher responses than Spring calves, with no significant differences between Spring-Winter or Fall-Winter groups although least squares means of Winter calves were similar to Fall calves.

Although female calves tended to demonstrate greater response than male calves, this was significant only for the peak response at 24 h post challenge (DTC24). This was after adjusting for birth weight differences.

Disease Relationships

Pneumonia occurred in 25% and diarrhea in 16% of the calves on trial. Least squares means of skin fold measurements for disease prevalence codes (Table 3) indicated no significant differences between treated and untreated animals for either pneumonia or diarrhea on the rate (DT240), intensity (DTC24, DTC48), persistency (DT4824) or duration (DT480) of the cutaneous DTH response to DNCB. Further, no differences were apparent for sick prevalence, which categorized animals as not-treated, treated for only one disease or treated for both diseases (Table 3). Similarly, the severity of pneumonia, diarrhea and sick were not significantly related to the DTH response, although most regression coefficients were positive (i.e. increased severity associated with increased response) for calves with pneumonia.

Non-specific inflammatory phase of the CMI may mask differences between high and low response groups. This may explain the

lack of significance between sick and healthy calves with respect to the cutaneous DTH response to DNCB in the present study. In addition, cutaneous DTH may reflect compartmentalized rather than systemic immunological status, and the lack of relationship between pneumonia or diarrhea and cutaneous DTH would not be unexpected. On the other hand, CMI may not make a significant contribution to the defense against neonatal pneumonia or diarrhea, given the predominantly extracellular nature of associated bacterial pathogens and lack of adaptation of the common viral agents to persist in macrophages.

Heritability Estimates

Paternal half-sib heritability estimates are given in Table 4. Although the standard errors on these estimates are high (because of the small numbers of sires and calves tested), some conclusions can be drawn.

Heritability estimates were moderate to high for inherent skin thickness (C0 and T0) and were moderate for response to vehicle (C24 and C48). Estimates for DTH response including inherent skin thickness plus vehicle (T24 and T48) were high, with the 48 h post challenge estimate being slightly larger than the 24 h estimate.

When magnitude of response is examined at 24 and 48 h post challenge (DTC24 and DTC48), heritability estimates are still moderate, even with the effects of inherent skin thickness and vehicle removed. The DTC48 response appears to be more highly heritable than that at DTC24 (peak). Along with a high heritability estimate, DTC48 may be a useful selection criterion because it may be a reflection of the sum of events including rate of onset, peak response and persistency of response.

Rate of onset to peak response (DT240) is moderately heritable, persistency of peak response (DT4824) is not heritable and the duration of response to 48 h (DT480) is moderately heritable. For the purpose of this study onset response (DT240) and/or duration of response (DT480) may be traits that more accurately assess the ability of calves to mount specific CMI to antigen challenge. It is, therefore, encouraging that the heritability estimates for these traits and DTC24 and DTC48 are moderate to high.

Genetic Correlations

Genetic and phenotypic correlations between all measurements of the cutaneous response to DNCB and between these and birth weight were computed. Body weight at birth (size) appears to be a component of inherent skin thickness (TO) but not of response to DNCB (all other skin thickness measurements). In general, the genetic correlations were larger than the phenotypic correlations. This implies that the relationships between rate, magnitude and duration of the DTH response to DNCB have a large genetic component, although the genetic correlations between inherent skin thickness (TO) and other DNCB skin responses tend to be low. Calves tend to hold their relative rank for DTH responses to DNCB whether measured at 24 or 48 h post challenge, and this seems to be genetically predetermined. The moderate to high heritability estimates for these response traits (Table 4) suggest potential response to selection, and the genetic correlations indicate that selection for either 24 h or 48 h DTH response will likely result in large correlated responses in both.

CONCLUSIONS

The study of marker traits may overcome the inherent difficulties of detection and quantification of disease incidence type traits for the genetic improvement of disease resistance in livestock. Accordingly, the objectives of the present study were to examine sources of variation in the DTH response using skin thickness after DNCB challenge treatment as the marker. Challenge dosages of DNCB given to previously sensitized calves resulted in substantial mean increases in double skin fold thickness which peaked at 24 h but subsided slightly by 48 h post challenge. Phenotypic and genetic correlations indicate relatively constant ranking of calves for 24 and 48 h measurements and further indicate that a large portion of each measurement is genetically determined. It was, therefore, encouraging that the heritability estimates were moderate to high,

even though the sample size of calves and sires was small. DTH response was affected by season of birth of the calves, but the reasons for this are obscure. The incidence and severity of neonatal pneumonia and diarrhea did not contribute significantly to DTH responses, likely due to the predominantly extracellular nature of the common infecting agents, and to the high compartmentalization of the specific skin response measured. The relationship between DTH skin response and diseases such as bovine rhino-tracheitis, brucellosis, salmonellosis and tick infestation should be investigated on a larger number of animals, since significance would give this DTH response the requirements of a successful marker trait on which to base sire selection for improved disease resistance.

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Table 1. Means of delayed type hypersensitivity response measurements to percutaneous challenge with dinitrochlorobenzene (DNCB) in 149 calves.

	Coded Response	Site of Measurement	Time post-challenge(h)	Mean response (mm skin)	SD	CV(%)
A.	C0	control	0	4.81	.88	18.20
	C24	control	24	4.98	.92	18.5
	C48	control	48	5.07	.97	19.10
	T0	test	0	4.86	.88	18.12
	T24	test	24	7.14	1.57	22.04
	T48	test	48	6.83	1.45	21.24
B.	DTC24	test-control	24	2.16	1.32	61.07
	DTC48	test-control	48	1.76	1.18	67.28
C.	DT240	test	24-0	2.28	1.31	57.66
	DT4824	test	48-24	-.31	.92	293.41
	DT480	test	48-0	1.97	1.22	62.26

Table 2. Least squares means or estimates for season, sex and birth weight effects on the delayed hypersensitivity response to percutaneous application of dinitrochlorobenzene (DNCB) in calves.

Independent Variable	n	Double skin fold thickness measurement[1] (mm) T0	T24	T48	DTC24	DTC48	DT240	DT4824	DT480
Season		(P=.001)[2]	(P=.0003)	(P=.003)	(P=.061)	(P=.048)	(P=.149)	(P=.436)	(P=.055)
Spring	57	4.22[b]	6.33[b]	5.59[b]	1.69[b]	1.05[b]	2.11	-.74	1.37[b]
Fall	45	5.00[a]	7.88[a]	7.27[a]	2.66[a]	1.95[a]	2.88	-.61	2.27[a]
Winter	47	5.02[ab]	7.42[ab]	7.49[ab]	2.39[ab]	2.11[ab]	2.40	.07	2.47[ab]
Sex		(P=.925)	(P=.143)	(P=.221)	(P=.045)	(P=.250)	(P=.123)	(P=.610)	(P=.203)
Male	69	4.74	6.90	6.54	1.86[b]	1.51	2.16	-.35	1.81
Female	80	4.75	7.52	7.02	2.64[a]	1.90	2.76	-.50	2.26
Birth weight (kg) (LS estimate)	149	(P=.041) .03	(P=.011) .07	(P=.015) .06	(P=.042) .05	(P=.291) .03	(P=.080) .05	(P=.619) -.01	(P=.130) .04

[1] T = test site measurement; DNCB applied in vehicle
C = control site measurement; vehicle only applied
D = a numerical difference (i.e. subtraction)
0 = prechallenge double skin fold thickness
24 = 24 h post challenge double skin fold thickness
48 = 48 h post challenge double skin fold thickness

[2] Probability associated with type III sums of squares F-test

[ab] Means with different superscripts within a variable sub-column differ at P<.05.

Table 3. Least squares means or estimates for disease prevalence and severity effects[1] on the delayed hypersensitivity response to percutaneous application of dinitrochlorobenzene (DNCB) in calves.

Independent Variable	n	Double skin fold thickness measurement[2] (mm) T0	T24	T48	DTC24	DTC48	DT240	DT4824	DT480
Pneumonia Prevalence		(P=.629)[3]	(P=.981)	(P=.645)	(P=.836)	(P=.710)	(P=.826)	(P=.564)	(P=.478)
Yes	37	4.66	7.20	6.96	2.30	1.87	2.54	-.24	2.30
No	112	4.74	7.21	6.82	2.23	1.75	2.46	-.39	2.08
Pneumonia Severity (P=.096)		(P=.539)	(P=.150)	(P=.064)	(P=.341)	(P=.102)	(P=.212)	(P=.722)	(P=.096)
LS estimate	37	.03	.14	.16	.08	.13	.11	.02	.13
Diarrhea Prevalence (P=.405)		(P=.832)	(P=.999)	(P=.511)	(P=.947)	(P=.426)	(P=.915)	(P=.379)	(P=.405)
Yes	24	4.68	7.20	7.01	2.26	1.93	2.52	-.20	2.32
No	125	4.72	7.20	6.77	2.28	1.68	2.48	-.43	2.05
Diarrhea Severity (P=.924)		(P=.843)	(P=.993)	(P=.849)	(P=.562)	(P=.648)	(P=.912)	(P=.792)	(P=.924)
LS estimate	24	-.02	.00	-.04	.12	.08	.02	-.04	-.02
Sick Prevalence		(P=.185)	(P=.495)	(P=.394)	(P=.910)	(P=.566)	(P=.906)	(P=.615)	(P=.566)
0	97	4.78	7.22	6.73	2.24	1.65	2.45	-.50	1.95
1	43	4.51	6.92	6.64	2.17	1.68	2.41	-.28	2.14
2	9	4.83	7.47	7.32	2.38	2.13	2.64	-.15	2.49
Sick Severity LS estimate		(P=.964)	(P=.368)	(P=.288)	(P=.365)	(P=.177)	(P=.362)	(P=.988)	(P=.284)
1	43	.01	.05	.06	.04	.04	.04	.01	.05
2	9	.01	.20	.20	.19	.22	.19	-.00	.19

[1] Prevalence was scored on an all (yes) or none (no) basis, and severity as the total number of days treated for a disease. The LS estimate for severity scores was based on the regression of double skin fold thickness measurements on severity after adjustment for prevalence of the disease.

[2] T = test site measurement; DNCB applied in vehicle
C = control site measurement; vehicle only applied
D = a numerical difference (ie. substraction)
0 = prechallenge double skin fold thickness
24 = 24 h post challenge double skin fold thickness
48 = 48 h post challenge double skin fold thickness

[3] Probability associated with type III sums of squares F-test

Table 4. Paternal half-sib heritability estimates of the delayed type hypersensitivity response to percutaneous application of dinitrochlorobenzene (DNCB) in young calves.

Response Measurement[1] (mm of skin)	Heritability[2] Estimate ± SE
CC0/	.612±.382
C24	.554±.367
C48	.545±.365
T0/	.525±.359
T24	.664±.394
T48	.676±.397
DTC24	.379±.316
DTC48	.564±.369
DT240/	.508±.355
DT4824	0
DT480/	.485±.348

[1] T = test site measurement; DNCB applied in vehicle
C = control site measurement; vehicle only applied
D = a numerical difference (ie. subtraction)
0 = prechallenge double skin fold thickness
24 = 24 h post challenge double skin fold thickness
48 = 48 h post challenge double skin fold thickness

[2] Heritability estimates were computed from a model that did not include birth weight. The heritability estimate for birth weight was calculated as h^2=.306±.300 and inclusion of this variable decreased heritability estimates of the inherent skin thickness measurements (C0/, C24, C48, T0/) and 48 h post challenge measurements (T48, DTC48, DT480/), while increasing 24 h post challenge measurements (T24, DTC24, DT240/).

CLONING AND EXPRESSION OF GENES FROM VIRULENT AND AVIRULENT STRAINS OF THE BOVINE PATHOGEN, UREAPLASMA DIVERSUM

N.L. Campbell, A.M. Gibbins and H.L. Ruhnke
Department of Animal and Poultry Science, University of Guelph and OMAF Veterinary Laboratory Services, Guelph (HLR).

SUMMARY

Ureaplasma diversum, a member of the class Mollicutes, is one of the smallest, simplest, self-replicating organisms known. This bacterium has been implicated in the etiology of diseases of the bovine reproductive, urinary and respiratory tract, and is of particular concern to the artificial insemination and embryo transfer industries. Ureaplasmas are extremely difficult and expensive to isolate from clinical samples; there are no tests available for distinguishing between virulent and avirulent strains.

The objective of this research is to use recombinant DNA techniques to isolate regions of DNA unique to virulent strains of U. diversum. From these regions of DNA, diagnostic probes are being constructed which will be used to detect the presence of virulent strains of U. diversum in semen and bovine tissue.

INTRODUCTION

Ureaplasma diversum can frequently be isolated from the preputial cavity of bulls as well as from raw semen samples. Although the extent of the effects of this organism on the bull is unknown, semen may be an important vehicle for transmission of infection to the cow (Fish et al., 1985). Jurmanova and Sterbova (1977) isolated mycoplasmas, including U. diversum, from bull semen and found a significant decrease in the motility of sperm from mycoplasma-infected samples. In a study of clinically normal bulls, U. diversum was found to be one of the most common mycoplasmal contaminants of semen samples collected for artificial insemination (A.I.) (Fish et al., 1985). These organisms can survive processing and freezing procedures and may be present in up to 14% of commercial semen samples available for A.I. (Onoviran et al., 1979). Because U. diversum is often isolated from normal animals as well as from those exhibiting symptoms of disease, a method must be developed to distinguish between putative pathogenic and non-pathogenic strains. Until such a method exists, all contaminated samples should be considered potentially pathogenic.

In semen samples processed for A.I., antibiotics are added to semen extenders to eliminate ureaplasmas, mycoplasmas, and other bacterial contaminants. Originally, penicillin alone was added but, when mycoplasmas were recognized as contaminants, a combination of lincomycin and spectinomycin (Lincospectin) was used along with the penicillin. Both of these are broad spectrum antibiotics that inhibit protein synthesis by acting on the 50S ribosomal subunit. When ureaplasmas were also identified as common contaminants of semen, minocycline (minocin) was added to semen extenders as well, because ureaplasmas are not suceptible to penicillin or lincospectin. Minocycline is a tetracycline-derivative and the antibiotic of choice for use against obligate intracellular parasites and mycoplasmas; it inhibits protein synthesis by binding to the 30S ribospmal subunit. Unfortunately, this antibiotic is spermicidal in semen extenders other than milk (Abreo, et al., 1979), and its activity (which is only bacteriostatic) is reversible upon dilution. Dilution may occur upon insemination of the cow or during development of the embryo as the antibiotics are absorbed into the surrounding tissue. Growth of the previously static contaminating organisms might then occur, with the possibility of a poor pregnancy outcome.

A new protocol for semen treatment has been developed which uses gentamicin (effective against walled bacteria), lincospectin (for mycoplasmas) and tylosin (for ureaplasmas). Tylosin is a macrolide antibiotic related to erythromycin, which is an antibiotic commonly used for treatment of ureaplasma infections of humans. This combination of antibiotics should be effective in all semen extenders (Shin et al., 1987).

Ureaplasma diversum may also play a detrimental role in the rapidly expanding embryo transfer industry. As for semen, the transferred embryo may be a means of spreading disease from one animal to another.

Wash procedures currently recommended for use in embryo transfer are not sufficient to remove contaminating U. diversum, which remains attached to the zona pellucida (Britton et al., 1988). Britton et al. detected ureaplasmas in the first wash fluids; by the tenth wash, no more organisms could be detected in the wash effluent but viable U. diversum could still be identified on the embryo.

Clearly a method is required for identification of pathogenic ureaplasmas that is specific and rapid, and does not involve culturing the organisms. Ureaplasmas have very specific growth requirements and are therefore difficult to culture. Even if an animal is shown to be ureaplasma-positive by culture, not all ureaplasma strains are virulent, so proof of virulence would depend on subsequent inoculation trials that are laborious, expensive and time-consuming.

Unique features which could distinguish virulent from avirulent strains must be found. No such research has been reported for ureaplasmas, but some unique features have been detected in virulent strains of certain mycoplasmas. For instance, an attachment protein (P1), required for pathogenicity, is altered in avirulent strains of Mycoplasma pneumoniae (Hu et al., 1984). Monoclonal antibodies directed at P1 do not recognize the P1-type protein produced by the avirulent strain. Ureaplasma diversum may also produce attachment proteins; as stated previously, this organism has been shown to adhere to the zona pellucida despite repeated washing (Britton et al., 1988).

Antibody and nucleic acid probes are now available for the detection and characterization of various mycoplasmas. Similar techniques can be used to develop diagnostic probes for Ureaplasma spp.

RESULTS AND DISCUSSION

Genomic libraries of an avirulent (4708-8) and two virulent (2312 and 8651#45FF) strains of U. diversum were constructed. The expression vector Lambda gt11 was originally selected for library construction but this method was unsuccessful, although repeated efforts were made using a variety of approaches. Use of the vector Lambda ZAP proved more successful. Libraries of one virulent strain (2312) and of the avirulent strain (4708-8) in Lambda ZAP were screened immunologically with antibodies raised against each of these strains. Several clones which appeared to be expressing ureaplasma proteins were selected from each library for further analysis. DNA from positive clones was isolated and examined to determine the size of the cloned ureaplasma DNA insert. Cell lysates of Escherichia coli hosts carrying the positive clones were examined by polyacrylamide gel electrophoresis and Western blotting to confirm the presence of ureaplasma proteins in the E. coli lysates. All of the isolated clones appeared to carry endogenous ureaplasma promotors implying that these promotors are functional in an E. coli system. The characterized clones are being tested for their use as diagnostic probes for U. diversum in general, but further work must be done in order to identify cloned ureaplasma DNA sequences that are unique to virulent strains alone.

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EFFECT OF BOVINE SOMATOTROPIN ON THE GROWTH RATE, HORMONE PROFILES AND CARCASS COMPOSITION OF HOLSTEIN BULL CALVES.

P.P. Groenewegen, B.W. McBride, J.H. Burton and T.H. Elsasser[1]
Department of Animal and Poultry Science, University of Guelph and [1]U.S. Department of Agriculture, Beltsville, Maryland

SUMMARY

A study was conducted to determine the effects of rBST on the growth rate, hormone profiles and carcass composition of preweaned Holstein bull calves. Injection of rBST resulted in increased average daily gains and feed intakes ($P<0.05$) compared to that of control animals. This increased rate of gain coupled with increased intakes resulted in identical feed conversion efficiencies between treatments. Fractional weights (organ weight/body weight) of selected organs and tissues were unaffected by rBST treatment. Carcass fat percentage was reduced in rBST-treated calves (28.1 ± 1.18% and 32.8 ± 1.84% for rBST and controls groups respectively, $P<0.05$) while no differences were seen for carcass DM, CP, ash or gross energy content. Plasma BST levels were higher ($P<0.0001$) in rBST treated calves while plasma IGF-1 levels were not different between treatments. Plasma IGF-1 concentrations correlated ($P<0.0001$, $r = 0.71$) with average daily gains. The results of this trial suggest that the growth responses induced by rBST in young calves (7 d to 3 months) mirrors that reported for older cattle.

INTRODUCTION

Bovine somatotropin is thought to elicit its growth promoting actions directly via insulin-like growth factor-1 (IGF-1)(12,13). Since all reported metabolically associated activities of IGF-1 are anabolic in nature (13) and IGF-1 levels increase in BST treated cows from 30 to 100% above controls (9,11,14), the theoretical improvements for the stimulation of growth are quite evident. Breier *et al.* (9,15) reported a highly significant relationship between plasma IGF-1 levels and average daily gain in 15 to 17 month old cattle. This relationship appears to be very closely regulated by the nutritional status of the animal (11). Although many of these studies do not deal directly with the use of BST, they describe relationships between hormone profiles and associated production parameters that may aid in the resolution of the effects of BST on growth.

Hormone profiles for lactating or growing animals at various nutritional and health states have been reported (9,11,16 to 21). Although most deal with nutritional manipulation, only a few deal with the neonate (16 to 18, 20), while none deals with chronic BST manipulation in the neonate. Therefore, the purpose of this study was to determine the effect of chronic BST administration on the growth rate, hormone profiles and subsequent carcass composition of Holstein bull calves from birth to three months of age.

METHODS and MATERIALS

Thirty Holstein bull calves were purchased from commercial dairy farms at one day of age and randomly assigned to one of three treatment groups: initial slaughter, control, and BST injection group. All calves received an intramuscular injection of 0.5 mL Dystocol and 1.0 mL of a Vitamin ADE injectable at the beginning of the experiment. Calves were housed in individual crates (1 X 2 m) with a 24 hour lighting regime. Calves were allowed a 5 to 7 day acclimation period prior to commencement of treatment at which time they were weighed, bled via jugular puncture and given a subcutaneous injection of either 0.1 mg BST/kg body weight per day (BST injection group; American Cyanamid Co., Princeton, NJ) or an equivalent volume of 0.9% saline (control group). The age of the calves at commencement of trial was 7 days. The treatment duration was 91 days.

Calves in the control and BST injection groups received whole milk at 10% of their weekly body weight until 28 days of age at which time the level was decreased to 8% body weight for the duration of the trial. Calves also received an 18% CP calf starter/grower (Mastermix, Maple Leaf Mills Ltd., London, Ontario) *ad libitum*. On a weekly basis, animals were weighed following the AM feeding and bled prior to injection. The milk intakes and

hormone administration levels were also adjusted at this time. Blood was collected in heparinized 10 mL Vacutainer tubes (Fisher Scientific, Don Mills, Ontario) and immediately placed on ice. Plasma was separated within 2 hours after blood collection by centrification at 2200 g for 20 minutes at 4°C. Plasma was then stored at -20°C until hormone analysis. Weekly composite samples of calf starter were frozen, ground through a 2 mm screen (Whiley Mill, A.H. Thomas Co., Philadelphia, PN.) and subsequently analyzed for crude protein, ether extract content, ash analytical dry matter and gross energy (AOAC, 1980). Milk samples were taken daily and pooled weekly and analyzed for protein, fat, lactose and solids-not-fat on a Multispec M Infrared Automated Milk Analyzer (The Multispec Corporation, York, England). Gross energy content of the milk was then calculated as previously described (22).

On day 91 of the treatment period, calves were weighed and bled prior to slaughter. Following slaughter all organs and viscera were weighed, ground through a 5 mm screen (Hobart Model 802, The Hobart Manufacturing Co., Don Mills, Ontario) and samples frozen. A carcass half was also frozen for chemical analysis while the remaining half was used for muscle dissection. The same procedure was used on the initial slaughter group to give baseline measurements to assess total accretion of carcass components over the treatment period. All organs, viscera and muscle samples were freeze dried for dry matter determination. Frozen carcass halves were ground through a 1.3 mm screen three times (Autio Model 801 B Meat Grinder, Autio Company, Astoria, OR.) and a representative aliquot sampled and freeze dried. Freeze dried carcass samples were ground in a Warning blender (Model 31BL46, Warning Products Division, Dynamics Corporation of America, New Hartford, CT.) to regain homogeneity and samples were analyzed for crude protein, ether extract, ash, analytical dry matter and gross energy (23). Blood samples were analyzed by radioimmunoassay for BST and IGF-1 as previously described (11,24).

All analysis involved the use of the General Linear Models (GLM) procedure of SAS (25) to determine differences between control and BST injected groups. Weekly body weight gains, average daily gains and hormone profiles were analyzed using repeated measures analysis (26,27). Logarithmic transformations were used in the analysis of hormone data to remove heterogeneity of variance. Regression analysis was used to analyze the relationship between average daily gains and plasma IGF-1 levels. Paired t-tests were used to determine differences between treatments for all organ and tissue data. Hormone profiles, feed intakes and organ data were analysed without the use of a covariate in the statistical model. Total body weight gains and average daily gains were analyzed using the animals initial weight as a covariate in the statistical model.

RESULTS

Daily BST injections resulted in increased body weight gains, average daily gains and final body weights compared to the control calves (Table 1). BST treated calves also had higher cumulative calf starter and milk intake. Control animals consumed 1.23 ± 0.07 kg DM/day while the BST treated calves consumed 1.49 ± 0.08 kg DM/day ($P < 0.05$).

Carcass weights between the two groups were not different. Daily BST injections resulted in a lower carcass fat percentage ($P<0.05$) compared to control calves while no differences were found for the other measured carcass components (Table 2).

Total accretion of carcass components, calculated as the difference between mass in final carcass and the average value of the initial slaughter group, and their respective efficiencies of conversion from feed intake are shown in Table 3. Calf starter was analyzed and contained 86.2% DM, 20.0% CP, 20.1% fat, 9.7% ash, 16.4 MJ GE/kg. No differences in total accretion of efficiency of nutrient utilization were found between treatment groups. All visceral organs and muscles were expressed on a body weight basis to normalize the tissues to body size. No differences were detected between the control and BST injected groups (Table 4).

The administration of 0.1 mg BST/kg body weight per day resulted in a significant increase ($P<0.0001$) in plasma BST levels over the 91 day treatment period. Although plasma

levels appeared to parallel each other for the first four weeks of treatment, a continuous divergence continued with BST injected calves having plasma BST levels reaching approximately 45 ± 2 ng/ml during the last 5 week period, while control calves averaged 6.4 ± 1 ng/ml during the same time period. Although plasma IGF-1 profiles were not different, a trend for increased plasma IGF-1 levels was detected over the entire treatment period for the BST treated calves. Plasma IGF-1 levels showed a continuous increase from 20 ng/mL initially to a final concentration of approximately 140 ng/mL by 91 days after treatment commencement.

DISCUSSION

The effect of chronic BST administration on growth rate was first demonstrated by Brumby (28) who found BST injections for a 12 week period resulted in a 10% increase in body weight gain over that of control animals. Live body weights of calves treated with BST in this experiment resulted in an 18 to 19% increase in body weight gain. Similar results have been reported in calves immunized against somatostatin where final body weight increased approximately 10% above control animals but since a small number of animals were used per treatment no significance was found (29). Increased growth rates have also been found with chronic treatment of pre-pubertal dairy heifers (21,30), growing steers (6) and pigs (8), while responses in sheep tend to be variable (4,31).

Increased voluntary intakes of animals treated with BST are largely seen in the lactating dairy cow (32) while most studies involving growing animals revealed either no increase (7) or a reduction in intake (8) thus resulting in increased feed conversion efficiencies. The increased total dry matter intake in calves treated with BST deviated from that normally seen in the older growing animals. This resulted in BST treated calves having identical rates of conversion as their control counterparts. Similar findings with young animals has been reported by Sandles and Peel (21), although these results were not significantly different. Vicini *et al.* (29), also found increased intakes in calves immunized against somatostatin which resulted in identical efficiencies of conversion (gain/feed) between the treatment groups.

The classical response to BST administration in growing animals is a decrease in carcass fat with a concurrent increase in carcass protein content (32). Several studies reported decreased carcass lipid and increased carcass protein in response to somatotropin treatment (4,6,8,31,33). However, Sandles and Peel (21) reported no change in carcass components in pre-pubertal dairy heifers treated with BST. Although BST administration resulted in a decrease in carcass fat percentage in bull calves, net accretion of fat in these calves was not different from controls (Table 3).

The apparent mode of action of BST, causing decreased lipid accretion, is to reduce adipose tissues responsiveness to lipogenic signals while increasing its sensitivity to lipolytic signals (1). *In vivo*, BST administration causes the mobilization of stored fatty acids resulting in subsequent increases in plasma non-estrified fatty acid (NEFA) levels which can then be utilized as an alternative energy source (34,35). Lipolytic challenges of epinephrine in BST treated cattle accentuate this lipolytic response (5). Furthermore, somatotropin has been shown to antagonize insulin-stimulated uptake of glucose by adipocytes *in vitro* (36). It is these reduced rates of lipid synthesis and increased rates (or abilities) of lipolysis in the presence of BST that are thought to result in the decreased accretion rates of fat in growing animals (1).

Protein accretion rates are enhanced in both ruminants (3,4,7,37,38) and in monogastrics (8,33,39) during the administration of their respective somatotropins. The mechanism of action of BST resulting in increased rates of protein accretion appears to be due to an alteration in the protein synthesis rate. Eisemann *et al.* (37) in steers, and Crompton and Lomax (40) and Pell and Bates (41) in sheep, attributed elevated rates of nitrogen retention in BST treated animals to increases in protein synthesis with no effect on protein degradation. In contrast, M^{c}Bride *et al.* (42) found no difference in fractional rates of protein synthesis between control and BST treated steers. *In vitro* the acute effects of BST appear to be due to its insulin-like action resulting in increased rates of amino acid

uptake and protein synthesis (43). Ernst and Froesch (44) demonstrated that *in vitro* BST induced cell proliferation was due to localized production of IGF-1 and that the addition of an IGF-1 antisera eradicated the response. Since the actions of IGF-1 are anabolic in nature (13) and their production is regulated by circulating BST levels, further research is required to determine their effects on protein accretion in the growing ruminant.

Expression of organs and carcass tissue weights as a percentage of body weight normalizes these tissues for direct treatment comparison. No differences existed in the fractional weights of the organs and tissues measured between BST and control treated calves (Table 4).

Injection of BST into veal calves resulted in a significant increase in plasma BST levels. Although hourly blood sampling was not undertaken in this study, the increased BST levels are similar to those reported in sheep (4). Sandles and Peel (21) reported baseline BST concentrations in BST injected heifers to be significantly greater than those of controls although they did not achieve the elevated levels reported in this study. In contrast, (7,37,45) reported increased plasma BST following BST injection but these values returned to control levels within 24 hours. Although increased levels of BST are seen following feeding (18,46) and may persist for approximately 3 hours, the consistent order of bleeding between BST-treated and control calves concludes that the measured concentration of BST in blood from BST injected calves was solely due to the injection of BST.

Blood plasma levels of IGF-1, although tending to be higher in the BST treated calves, were not significantly greater than these of the control calves. Similar results have been reported in BST treated animals (20,21). However, the increasing plasma IGF-1 concentrations detected in this experiment were not seen in the aforementioned trials. Calves in the present study were allowed *ad libitum* access to a calf starter plus their respective daily milk intakes while in other trials (20) calves of received only milk in their diets. Animals fed diets with high protein and energy density are observed to have higher circulating plasma IGF-1 levels (11,15,19). Therefore, the difference in plasma IGF-1 concentrations seen between this trial and others (20) may thus be explained by differences in nutrient intake.

Regression analysis of plasma IGF-1 concentration on average daily gain resulted in a highly significant linear relationship ($P<0.0001$, $r = 0.71$) which is similar to that found by (9) with steers on different planes of nutrition. Aston *et al.* (49) reported similar correlations between plasma IGF-1 concentrations and average daily gains in growth hormone treated hypophysectomized rats. The ability to correlate a circulating hormone such as IGF-1 with a production related parameter such as average daily gain in growing ruminants should be more closely researched to allow possible selection of superior animals by mere evaluation of endogenous hormone profiles.

In conclusion, the effect of exogenous BST administration in veal calves elicits the same growth promoting response as that seen in the more mature ruminant. The increased rates of gain in veal calves, unlike that in steers or pigs, is compensated for by increased dry matter intakes which resulted in no increase in efficiency of feed utilization. No differences where seen in non carcass components but carcasses from BST treated calves had a lower percentage of fat compared to that of the control calves. Although a significant relationship exists between circulating IGF-1 concentrations and average daily gain the circulating levels of IGF-1 in plasma appear to be maximally expressed by endogenous BST levels over the time frame considered. Maturation of the liver somatotropic axis by twelve weeks of age may be responsible for the apparent lack of plateau in the IGF-1 profiles in calves treated with BST.

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Table 1. The Effect of BST Injections on Body Weight Changes and Average Daily Gain in Veal Calves.[1]

PARAMETER	CONTROL		BST	
Number per Treatment	9.0		10.0	
Initial weight (kg)	43.10	1.39	45.00	1.71
Final Weight (kg)	119.10	6.31	135.30	4.70[a]
Total Gain (kg)	76.10	5.92	90.40	3.68[b]
ADG (kg/day)	0.84	0.07	0.99	0.04[b]

[1] Values are MEANS SEM.
[a] Means significantly different from controls $P<0.10$.
[b] Means significantly different from controls $P<0.05$.

Table 2. The Effect of Daily BST Injections on Carcass Components in Veal Calves.[1]

COMPONENT	CONTROL		BST	
Carcass weight (kg)	63.7	3.74	67.8	2.81
Dressing Percentage	56.8	0.90	54.9	0.97
Carcass Dry Matter (%)	38.5	1.69	35.6	0.55
Carcass Crude Protein (%)	60.1	0.88	61.1	0.99
Carcass Fat (%)	32.8	1.84	28.1	1.18[a]
Carcass Ash (%)	15.3	0.45	15.7	0.41
Carcass Energy(MJ GE/kg)	22.0	0.42	21.7	0.17

[1] Values are MEANS SEM.
[a] Means significantly different from controls ($P<0.05$).

Table 3. The Effect of Daily BST Injections on Carcass Component Accretion and on Efficiency of Nutrient Conversion in Veal Calves.[1]

COMPONENT	CONTROL		BST	
i) Accretion				
Protein (kg)	9.62	1.10	9.63	0.68
Fat (kg)	6.91	0.99	5.57	0.72
Gross Energy (MJ)	401.00	41.7	384.70	32.7
ii) Efficiency[2]				
Protein (gain/intake)	0.35	0.05	0.30	0.02
Energy (gain/intake)	0.16	0.02	0.13	0.01
Dry Matter (gain/intake)	0.33	0.03	0.31	0.02
Gain (BW gain/intake)[3]	0.62	0.04	0.62	0.03

[1] Values are MEANS SEM on a 100% dry matter basis.
[2] Conversions based on Accretion (kg)/Intake (kg).
[3] Feed conversion based on whole body weight gain/Dry Matter Intake.

Table 4. The Effect of Daily BST Injections on Tissue Weights in Veal Calves.[1]

TISSUE	CONTROL		BST	
Hide (fresh kg/BW)[2]	6.50	0.31	6.38	0.24
Trachea (fresh kg/BW)[2]	0.14	0.02	0.12	0.02
Perirenal Fat	0.44	0.05	0.33	0.05
Spleen	0.15	0.02	0.13	0.01
Thymus	0.07	0.02	0.11	0.03
Heart	0.17	0.02	0.15	0.01
Kidney	0.12	0.01	0.11	0.01
Liver	0.57	0.03	0.52	0.02
Lungs	0.23	0.02	0.24	0.01
Stomach	0.62	0.04	0.64	0.02
Small Intestine	0.46	0.03	0.47	0.02
Large Intestine	0.29	0.02	0.28	0.01
Biceps femoris	0.33	0.03	0.30	0.01
Semitendinosus	0.36	0.02	0.33	0.01
Semimembranosus	0.11	0.01	0.10	0.01
Longissimus dorsi	0.23	0.02	0.21	0.01

[1] Means reported are expressed as kg dry weight/kg slaughter BW.
[2] Means expressed as kg fresh weight/kg slaughter BW.

THE BIOACTIVITY OF MILK FROM BST-TREATED COWS

P.P. Groenewegen, B.W. M°Bride, J.H. Burton and T.H. Elsasser[1]
Department of Animal and Poultry Science, University of Guelph and [1]U.S. Department of Agriculture, Beltsville, Maryland

INTRODUCTION

Development of DNA technology to produce bovine somatotropin (BST) has potentiated production increases in dairy cows that would have traditionally required numerous generations of genetic selection to acquire. Furthermore, the production of milk from cows treated with hormones has raised concern with regards to the wholesomeness of milk. However, limited published information exists regarding BST and insulin-like growth factor-1 (IGF-1) residues in the milk from BST-treated cows. BST and IGF-1 are known to exist naturally in milk (1 to 10). Hart *et al.* (4) reported levels of BST in milk from BST treated cows to be only slightly higher than those of non-treated cows. Also, no published information is available regarding the effect of pasteurization of milk on subsequent BST residues. Although initial concerns focused on milk BST residues, current interest is also directed toward milk IGF-1 levels following BST administration. Since bovine and human IGF-1 possess the same sequence homology (11) the effect of increased IGF-1 on the potential bioactivity of milk from BST-treated cows remains to be answered. Thus, the purpose of this experiment was to determine if milk from BST-treated cows contains any biological activity after oral ingestion.

METHODS AND MATERIALS

Hypophysectomized (HYPOX) male Sprague-Dawley rats (28 days old, 74 ± 0.4 grams) were purchased from Charles River Canada Inc. (Montreal, Quebec) and maintained in accordance with the Guideline to the Care and Use of Experimental Animals (Canadian Council on Animal Care, Ottawa). Rats were housed in wire bottom cages with 12 hour lighting (commencing at 0600 h) with room temperature maintained at 22-25°C. Rats were housed for a total of twenty-one days: days 1-6 were an acclimation period while days 7-21 represented the treatment period. Animals were offered a commercial rat chow (Ralston Purina Canada) and water *ad libitum*. Rats were weighed daily. Rats that maintained an average daily gain of -1 to 2 grams/day during the acclimation period were deemed completely hypophysectomized (12) and thus retained for the experiment.

Rats that successfully met the selection criteria were then randomly assigned to one of six treatments. Milk, obtained from the University dairy herd, was sampled from three non-BST-treated (82.3 ± 17 days postpartum) and three BST-treated cows (10.6 mg BST/day, American Cyanamid, Princeton, NJ., 78 ± 6 days postpartum) and pooled within treatment prior to feeding. To assess the effects on growth parameters of milk from BST-treated cows, milk from non-treated cows (TRMT 1) was used as a negative control from which the remaining five treatments were compared. TRMT 2 compared directly the effect of oral ingestion of BST. This was accomplished by adding BST to milk from non-treated cows. BST was added at a level of 500 ng/ml. This was approximately 500 times the hormone levels previously reported for cows milk (4). TRMT 3 consisted of milk from BST-treated cows while TRMTs 4, 5 and 6 were pasteurized control milk, pasteurized control milk spiked with BST and pasteurized milk from BST-treated cows, respectively. The experiment was conducted over three time periods.

A second experiment was conducted to achieve oral intakes of BST greater than that given systemically. Four milk treatment groups were fed in an identical manner as described below. The four treatment groups were; milk from control cows (1), pasteurized milk from control cows (2), milk from control cows spiked with 160 ug BST/mL (3) and pasteurized control milk supplemented with 160 ug BST/mL.

Milk was pasteurized using a Safgard Home Milk and Cream Pasteurizer (Model P-3000, Janesville, Wisconsin). Pasteurization consisted of heating milk to 69-71°C for 25-30 minutes followed by rapid cooling. Animals were fed their respective treatments at 10% of their daily body weights in three equal feedings at 0700, 1200 and 1800 h daily. Composite milk samples were analyzed for their components

(fat, protein, lactose and solids-not-fat) using a Multispec M Infrared Automated Milk Analyser (The Multispec Corporation, York, England) and all milk was fed on an isocaloric basis based on calculations from the major components (13). Rat chow intakes were recorded weekly. Milk samples were subsequently analyzed for BST and IGF-1 using their respective double antibody radioimmunoassays (1,3) as modified by Groenewegen et al.(14).

On day fourteen of the treatment period the rats were euthanized (using methoxyfloran; Metaphan, MTC Pharmaceuticals, Mississauga, Ontario) at which time the liver and thymus were dissected from the carcass, weighed and subsequently dried at 65 C for 48 h in a drying oven to achieve dry weights. A tibia was also dissected free of all soft tissue, the proximal end split along the midsaggital plane and stained using the silver nitrate technique (15) as modified by Li (16). The width of the epiphyseal growth plate was then measured using a calibrated eyepiece micrometer.

To assess the ability of a subcutaneous injection of BST to elicit a response in the HYPOX rat, a second group of rats were housed and cared for in the exact manner as that described above. Rats were injected with either 0, 5, 20 and 80 ug BST/day (17) for the same duration as the oral ingestion experiment (14 days). Interscapular injection volumes of 0.2 ml were administered using a 1 ml tuberculin syringe and 26 gauge 9.5 mm needle (Fisher Scientific, Don Mills, Ontario). All animals were fed milk from control cows in the same manner as that described above. All growth parameters considered in the first experiment were also measured in this experiment.

All statistical analyses were computed using the General Linear Models procedure of the Statistical Analysis Systems (18). T-tests were used to determine differences between treatments. Data are reported as significant if $P<0.05$.

RESULTS

Average daily gains, width of the epiphyseal growth plate and average daily intakes of rat chow of rats fed the milk treatments (milk at 10% their daily body weight for a fourteen day treatment period) are shown in Table 1. No significant differences were detected ($P>0.05$) between treatments for any of these variables. Since no differences occurred between the *ad libitum* rat chow intakes among the six groups the only differences between the treatments was that of the respective milk treatment.

To normalize organ weights with respect to body size, all organs are expressed as grams dry matter per gram live body weight (fractional weights) using the body weight obtained at the 0600 weighing on day 14 of treatment. Fractional weights for the liver and thymus are shown in Table 2. The effect of the preplanned contrast, pasteurized versus non-pasteurized, revealed the fractional weights of the livers from rats fed non-pasteurized milk to be significantly heavier than their counterparts from the pasteurized milk-fed rats ($P<0.001$). No differences between treatment groups were seen with respect to fractional weights of the thymus.

Table 3 shows milk BST residues prior to and following pasteurization. The effect of pasteurization of the control milk spiked with BST was significant and represents a reduction in immunoreactive BST by 85 to 90%. The levels of BST in milk from BST-treated cows were not significantly different from that of control cows, and while pasteurization reduced the levels of BST in milk from BST-treated and control cows, the reduction was not significant. Milk levels of IGF-1 in both control and milk from BST-treated cows was below the lower limits of detection in the IGF-1 assay used, namely 2.9 ng IGF-1/ml. Due to the levels being below that of the assay, the effect of pasteurization on milk IGF-1 levels could not be determined.

Oral intakes of 1.3 ± 0.03 mg BST/day had no effect on average daily gains, width of the epiphyseal growth plate (Table 4) or on fractional weights of the liver and thymus (Table 5).

Daily rat chow intakes for the four injection groups were 5.76 ± 0.22, 6.47 ± 0.30, 6.65 ± 0.23 and 7.25 ± 0.29 grams per day for the 0, 5, 20 and 80 ug BST/day injection levels, respectively. Only the 80 ug BST/day injection

level resulted in significantly greater rat chow intakes, but daily milk intakes, fed on a constant percentage body weight, increased with increasing level of injection. A dose dependent response in both the tibial growth and the rat body weight gain was evident. The dose protocol used in this experiment did not result in a maximal response in either the tibial growth or the body weight gain. Unlike the first experiment, systemic administration of BST elicited a significant change in both the fractional weight of the liver and thymus (Table 6). The lower level of BST administration (5 ug BST/day) did not alter the fractional growth of the liver but increasing the dose of BST resulted in proportionally more body growth compared to liver growth. In contrast, the thymus showed an increase in fractional weight with higher BST doses (Table 4). Since the response to systemic BST administration was dose dependent, the injection group served as an adequate positive control for the first experiment.

DISCUSSION

Our results suggest that no biologically active proteins and/or fragments of BST or IGF-1 were absorbed as evidenced by a lack of a tibial growth response. Similarly, Malven *et al.* (21) found no absorption of milk prolactin into the blood from milk fed rats and calves. Since bovine prolactin and BST have approximately the same M, (approximately 22 000 Da) the probable reasoning for this lack of any biological activity after oral administration is digestion of the proteins to amino acids or small peptide fragments (19), all of which seem to possess no growth promoting activity post-adsorptively.

Although the absorption of protein fragments from the gastrointestinal tract (19) and the possible importance of intact protein absorption for the ontogeny of the digestive system (10,20) is beginning to be understood, no growth promoting activity was found in milk from BST-treated cows or from BST administered orally. Similar results using the HYPOX rat body-weight-gain bioassay were reported by Seaman et al. (12), but the sensitivity of the body-weight-gain bioassay is only about one-twentieth that of the tibial growth bioassay (16) employed in this study. Since no response was detected using the tibial growth assay, it was not surprising to see no difference in average daily gains among the six treatment groups.

The use of the liver and thymus as specific organs to monitor the effect of BST and IGF-1 in the HYPOX rat is well documented (16,22,23). The results of the systemic administration of BST to the HYPOX rat reveals the cross species biological activity exerted by BST on the HYPOX rat. Large enough quantities of IGF-1 were unavailable for oral challenges although the effect of systemic administration of IGF-1 in the HYPOX rat is known (22,23) and the response appears to mirror that of BST.

Width of the epiphyseal growth plate increased in a dose dependent manner at each dose level of BST. Although subcutaneously administered BST led to a growth response at 5 ug/day an equivalent dose given orally produced no response. Similar findings occurred at oral intakes of 1.3 ± 0.03 mg BST/day and as high as 4 mg BST/day (12). Guler *et al.* (22) found a greater growth of the epiphyseal plate in response to human growth hormone (hGH) than for human IGF-1 (hIGF-1). This suggested that hIGF-1 was less potent in the rat than rat IGF-1 for stimulating epiphyseal plate growth. In contrast, human GH given subcutaneously causes increased levels of endogenous IGF-1 in the rat. Rat IGF-1 is 2-3 times more potent than hIGF-1 (24). BST may also act directly on bone growth in the HYPOX rat by regulating IGF-1 m-RNA, thus regulating local IGF-1 production. This localized production of IGF-1 may then potentiate a growth response (25). One further explanation why hGH resulted in a greater response than hIGF-1 is the existence of IGF-1 in circulation. A systemic dose of IGF-1 would result in large quantities of free IGF-1 in circulation while hGH would result in IGF-1 circulating in its native form, bound to its binding protein. The binding protein is hypothesized to be a promoter of IGF-1 action (26), therefore bound IGF-1 would elicit a greater growth response than free IGF-1. Thus, systemic BST and IGF-1 can elicit growth responses in the HYPOX rat. However, based upon our study, oral ingestion of either BST directly or milk from BST-treated cows eradicates this activity.

Similar results are shown for the effects of BST on the liver and thymus (Table 6). Fractional weights of the liver decreased with increasing systemic dose of BST while that of the thymus increased. Guler *et al.* (22) reported that hGH was less potent than IGF-1 in restoring fractional tissue weights but levels of the hormone injected were two to three times the levels injected in the present experiment. The lower doses of BST administered in this study may thus indicate that the production of IGF-1 in response to BST did not reach levels required to maximally stimulate organ growth.

The contrast comparing groups fed pasteurized versus non-pasteurized milk was not designed to consider the effect of pasteurization on milk *per se* but to examine the overall effect of pasteurization on milk hormone residues. Since milk BST residues are reduced due to pasteurization (Table 3) it may lead one to erroneously believe the lower levels of BST residues in milk resulted in the lower fractional liver weights. If this was the case then the fractional weights of the livers from the rats fed pasteurized BST spiked control milk should be higher than those of the other two pasteurized groups since hormone levels were significantly higher than those of the other pasteurized groups (Table 3). The fractional weights of the livers from the rats fed pasteurized milk were not different from one another, thus precluding the effect of increased hormone residue on fractional liver weights. The effect of feeding pasteurized milk to rats on liver lipid has been examined and resulted in decreased liver lipid contents in rats fed pasteurized milk (27). This reduction in liver lipid content combined with slight alteration in digestion of milk proteins following pasteurization (28) may have led to the slight reduction in the fractional liver weights from the rats fed the pasteurized milk.

In conclusion, it is apparent that milk from BST-treated cows contains hormone residues that are not different from those found in control cows. Furthermore, pasteurization effectively reduced the immunoreactive quantities of BST in milk. Finally, no growth promoting activity was detected when BST or milk from BST-treated cows was fed orally.

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Table 1. The Effect of Feeding Treated Milk on Average Daily Gains (ADG), Width of the Epiphyseal Growth Plate and Daily Feed Intake in the HYPOX Rat.[1]

TREATMENT	N	ADG (grams/day)	WIDTH (um)	INTAKE (grams/day)
Control Milk	34	0.17 ± 0.04	163 ± 3.0	5.6 ± 0.2
BST Spiked Milk	33	0.20 ± 0.04	164 ± 3.1	6.1 ± 0.2
BST Milk	30	0.15 ± 0.05	165 ± 3.3	5.7 ± 0.2
Past. Control Milk	32	0.22 ± 0.05	161 ± 3.2	5.8 ± 0.2
Past. BST Spiked Milk	30	0.23 ± 0.05	162 ± 3.3	5.9 ± 0.2
Past. BST Milk	30	0.20 ± 0.05	166 ± 3.3	5.8 ± 0.2

[1] Values reported are LSMeans ± SEM.

Table 2. The Effect of Feeding Treated Milk on Fractional Weights of the Liver and Thymus in the HYPOX Rat.[1]

TREATMENT	LIVER[b]	THYMUS
	(g DM/g Body Weight)	
Control Milk	1.380 ± 0.020	0.049 ± 0.002
BST Spiked Milk	1.357 ± 0.020	0.045 ± 0.002
BST Milk	1.340 ± 0.021	0.050 ± 0.002
Past. Control Milk	1.298 ± 0.020	0.046 ± 0.002
Past. BST Spiked Milk	1.299 ± 0.021	0.047 ± 0.002
Past. BST Milk	1.258 ± 0.021	0.052 ± 0.002

[1] Values are LSMeans ± SEM.
[b] Preplanned contrast, pasteurized versus non-pasteurized, significant ($P<0.001$).

Table 3. BST Residues Prior to and After Pasteurization in Milk from Control, BST-Treated and Control Spiked Milk.[1,2]

TREATMENT	BST (ng/ml)
Control Milk	3.26 ± 1.68
Control BST Spiked Milk	481.90 ± 53.0[a]
Milk from BST treated cows	4.22 ± 1.87
Pasteurized Control Milk	2.73 ± 0.47
Pasteurized BST Spiked Milk	43.4 ± 2.85[b]
Pasteurized BST Milk	3.38 ± 1.11

[1] Values reported are MEANS ± SEM in ng/ml, N=12 per treatment.
[2] IGF-1 residues were below 2.9 ng/ml for all milk samples analyzed.
[a,b] Means in the same column with different superscripts differ ($P<0.0001$).

Table 4. The Effect of Feeding BST Treated Milk on the Width of the Epiphyseal Growth Plate and Average Daily Gain in the HYPOX Rat.[1]

TREATMENT	N	TIBIA WIDTH (um)	ADG (g/day)
Control Milk	10	152.85 ± 5.74	0.19 ± 0.10
Past. Control Milk	11	151.32 ± 5.08	0.29 ± 0.07
160 ug BST Spiked Milk	11	152.22 ± 4.21	0.31 ± 0.08
Past. 160 1g BST Milk	9	153.50 ± 3.73	0.16 ± 0.08

[1] Values reported are MEANS ±SEM.

Table 5. The Effect of Feeding BST Treated Milk on Fractional Weights of the Liver and Thymus in the HYPOX Rat.[1]

TREATMENT	LIVER (g DM/g Body Weight)	THYMUS (g DM/g Body Weight)
Control	1.354 ± 0.014 [a]	0.036 ± 0.004
Past Control	1.289 ± 0.029[ab]	0.044 ± 0.002
160 ug BST Spiked	1.323 ± 0.020 [a]	0.044 ± 0.003
Past 160 ug BST	1.226 ± 0.021 [b]	0.043 ± 0.002

[1] Values reported are MEANS ± SEM.
[a,b] Means with different superscripts differ (P<0.05).

Table 6. The Effect of BST injection on Fractional Weights (%) of the Liver and Thymus in the HYPOX Rat.[1]

TREATMENT	LIVER (g DM/g Body Weight)	THYMUS (g DM/g Body Weight)
0 ug BST/day	1.380 ± 0.029[a]	0.040 ± 0.005[a]
5 ug BST/day	1.432 ± 0.028[a]	0.054 ± 0.004[b]
20 ug BST/day	1.295 ± 0.017[b]	0.054 ± 0.002[b]
80 ug BST/day	1.182 ± 0.019[c]	0.070 ± 0.002[c]

[1] Values are MEANS ± SEM.
[a,b,c] Means in the same column with different superscripts differ (P<0.05).

FEEDING STRATEGIES AND THEIR EFFECTS ON PERFORMANCE OF LACTATING DAIRY COWS.

G.K. Macleod, D.G. Grieve and N.J. Lewis
Department of Animal and Poultry Science, University of Guelph

SUMMARY

The effects of frequency of feeding concentrates, the addition of hay and the sequence of hay and grain feeding in hay crop silage (HCS) based diets of lactating Holstein cows were studied. Twelve primiparous and twenty multiparous cows were assigned to eight 4x4 latin squares. Data were analysed from the last 2 weeks of each 4 week period. Treatments were: (1) concentrate offered 2x/d, no hay, (2) concentrate offered 6x/d, no hay, (3) hay offered for 2 hours before concentrate feeding, and (4) hay offered .5 hour after concentrate and available for 2.5 hours before HCS. In each treatment HCS was offered ad libitum 2x/d following other feeds. Concentrate allowance was adjusted weekly to maintain a 60:40 concentrate : forage ratio. Voluntary consumption of hay averaged 1.7 kg in each of two feedings per day, in treatments 3 and 4. Frequency of feeding and sequence of feeding hay and concentrate had no effect on any production measurement, whereas the addition of hay to diets increased total feed intake, milk, protein and lactose yields. Hay decreased acetate, isobutyrate and isovalerate of rumen fluid and increased propionate, butyrate and valerate. Rumen ammonia and pH were not affected by treatments. This study did not yield evidence of beneficial effects of any feeding strategy examined on fat test of lactating cows.

INTRODUCTION

As dairy cows increase milk production, it is necessary to feed higher levels of concentrate in order to minimize negative energy balance. At the upper limits of recommended concentrate feeding (ex. 60:40 concentrate : forage ratio), some drop in milk fat test is usually expected. Maximal feed intakes and fat tests are especially difficult to maintain when cows are provided with high quality hay crop silage and no hay is offered. Feeding strategies based on concepts for improved rumen function have been developed and are commonly recommended. For instance some German research showed improved rumen pH, acetate to propionate ratio in rumen fluid and higher milk fat tests with 6 times (6x) daily feeding of concentrates compared with 2x. Several types of equipment permit frequent feeding of concentrates with minimal labour input.

A second approach, namely feeding of some hay along with free choice hay crop silage (HCS), has been shown to increase total feed intake and milk yield, but not fat tests, at the University of Guelph.

A third feeding strategy, which suggests that feeding of hay before concentrate rather than after, will improve rumen function, milk yield and fat test. While seeming to make good sense and being widely accepted in the field, there is very little research to support the practice.

Therefore, this study was undertaken to provide some data on the effects of these three strategies on rumen function and on performance of lactating cows.

PROCEDURE

Twelve primiparous and twenty multiparous Holstein cows were assigned to eight 4x4 latin squares. That is, there were four experimental treatments and four periods of four weeks duration so that each cow experienced each treatment. Cows were on a standard early lactation feeding program before moving to treatments at 4 to 7 weeks postpartum. Treatments were as follows:

2x - concentrate offered twice daily (2x). Hay crop silage (HCS) was fed one hour after concentrate. No hay.

6x - concentrate was offered 6x daily. HCS was offered at times similar to that in the first treatment. No hay.

HB - hay was offered 2 hours before concentrate, twice daily. HCS was offered free choice as in first two treatments.

HA - hay was offered .5 hour after concentrate, twice daily. HCS was offered 2.5 hours after hay, also 2x.

Cows in the last two treatments received a mixed hay (alfalfa predominating) - as much as they would eat in the time allowed. This was found to be approximately 1.7 kg (as fed basis) at each of two feedings. Hay crop silage was of high quality - i.e., approx. 45% dry matter and 23% protein. It was offered ad libitum.

The concentrate was a commercial dairy ration, commonly available in Ontario and consisted chiefly of shelled corn, barley, and wheat shorts with soybean meal, meat meal, and distillers grains providing supplemental protein of a desirable degree of rumen degradability. The ration was balanced for minerals and vitamins. The concentrate allowance was adjusted as needed weekly to maintain a 60:40 concentrate : forage ratio on a dry matter basis.

Total daily dry matter intake was calculated, daily milk yield was recorded, and twice a day sampling of milk for two days per week was analyzed for fat, protein and lactose content. Cows were weighed at the beginning and end of each period. Feed intake and milk data from the last two weeks of each period was analyzed by standard statistical procedures and orthogonal contrasts determined for significance as follows: 2x vs. 6x feeding, hay vs. no hay, and hay before vs. hay after concentrate feeding. Similarly, four rumen fistulated cows formed any additional latin square and rumen pH, ammonia level, and molar proportions of volatile fatty acids of rumen fluid collected during the last week of each period were determined and analyzed statistically as above.

RESULTS

The chief findings of this study are summarized in Tables 1 to 3. Live body weight of cows was not affected by treatment during the four week periods. Both first lactation and older cows were gaining in weight, on average, with heifers gaining more than cows, as expected. The addition of hay to HB and HA treatments resulted in significantly greater roughage, concentrate and total DM intakes. Intake of hay was approximately 3.1 kg per day (DM basis) and, as expected, reduced silage intake but not total forage intake. Concentrate feeding frequency and hay-concentrate sequence did not affect intakes significantly ($P>0.05$).

Actual milk yield, but not 4% fat-corrected milk yield, increased in response to hay feeding. Surprisingly, fat test actually dropped when cows were offered hay. A slight increase in lactose content of milk resulted when feeding frequency of concentrate was increased to 6x. Otherwise, feeding frequency had no effects on performance. As a result of increased milk yield with no effect on content of protein and lactose, yields of protein and lactose increased in response to hay feeding. Sequence of feeding hay and concentrate had no significant effect on lactation in this study.

Rumen parameters were not affected by frequency of concentrate feeding. These results are averages of thirteen samples per day and diurnal variations have not yet been assessed. Hay feeding significantly decreased molar proportions, on average, of acetate, isobutyrate, and isovalerate, while increasing, on average, the proportion on propionate, butyrate and valerate. Feeding hay after concentrate rather than before significantly increased the mean proportion of isobutyrate while significantly decreasing the mean proportion of valerate. Rumen pH and ammonia -N in liquid were not affected by treatments.

Throughout the experiment, first lactation cows responded similarly to multiparous cows.

DISCUSSION

Heavy grain feeding of dairy cows usually results in reduced pH in the rumen and reduced acetate : propionate ratios in rumen fluid. A consequence of these changes is lower fat content of milk. It has been suggested that by spreading the grain feeding over more than two feedings per day, pH and acetate : propionate ratio may not be so depressed. Kaufmann et al. (1980) have reported that by feeding 6x per day, rumen pH was higher, acetate : propionate ratio was higher and milk

fat test was greater than when cows received concentrate twice daily. British work has been cited in support of these findings. However, closer inspection of the trial of Sutton et al. (1986) shows a drop in pH when cows were fed >4x/day, and no significant difference in volatile fatty acid proportions in any of their experiments. On a 60:40 concentrate : forage diet they reported a non-significant decrease in milk fat test going from 2x to 5x feeding per day. One of two experiments on 2x vs. 6x gave an increased milk fat test and no effect in the other. Burt and Dunton (1967) compared 2x vs. 10x/d and saw no effect on fat test but an increase in lactose percent on 10x (an effect we observed but cannot explain). Yet another British scientist, Johnson (1979, 1980) reported only a non-significant increase in milk fat test where cows were fed concentrates 5x. Gill and Castle (1983) reported no significant effects of 2x vs. 4x vs. 22x daily on feed intake, milk yield and a non-significant increase in fat test. American workers Yang and Varga (1989) have just reported that cows fed a 55% corn silage, 45% corn-soy diet, 1x, 2x or 4x, increase yield of protein significantly but saw no difference in yield of milk, percent fat, dry matter intake, rumen pH, or proportions of VFA. Our results appear to agree with most others. However, certain factors may affect response. These include stage of lactation, level of production, level of grain feeding and physical form and fibre levels of both forages and the concentrates.

The increases in feed DM intake and milk yield when hay was fed are consistent with two experiments of Beauchemin (1988) at Guelph. The feeding of hay in the HCS based rations increased DM of the total rations by six percentage units. This change in DM is not expected to increase feed intake at the dry matter levels of rations in this study (72% for 2x and 6x). Digestibility of ration nutrients was not determined but an improvement could account for higher intakes and better milk yields. The significant fat test depression associated with hay feeding in this experiment was not expected though the rumen fatty acid results are consistent with that observation. It may be helpful to note that most of the fat test depression was associated with the hay after concentrate treatment and, that the mean for the hay before concentrate treatment was quite close to means for the first two treatments. Beauchemin did not see a fat test effect when hay was fed before concentrates in her studies.

Feeding hay before concentrates, particularly in the morning, has been widely recommended for herds in which there is a milk fat test depression. It would seem logical that such a practice might minimize the drops in rumen pH and acetate : propionate ratio frequently observed following high level of grain consumption. While independent research on the rumen effects has been reported, the consequential effect on milk fat test has rarely been studied. A complex series of experiments conducted at Uppsala, Sweden (Johnson, 1980) do suggest a benefit for feeding hay two hours before concentrates plus frequent feeding of concentrates plus more roughage. The lack of agreement between Swedish and Guelph results may be due to my contrasting conditions of the experiments.

Finally, a number of conditions in a particular herd may determine whether feeding strategies will improve milk yield, fat test etc. Cows may be most responsive very early in lactation, and/or at very high milk production levels, and when fed high levels of concentrates, the physical characteristics of both concentrates and roughages, and their fibre levels may be important contributing factors. Hay feeding and sequence of hay and concentrates may also be influenced in their effects by quality of hay relative to the silage, and by intervals that cows have to consume each feed (in particular, hay).

The increased feed intake and milk yield associated with hay feeding appears to be fairly consistent. The advantage of feeding some hay in an otherwise silage-based feeding program needs to be balanced against the possible inconvenience of providing some hay before concentrates. Consideration may want to be given to alternate feeding systems such as total mixed rations as well as to combinations of feeding strategies.

ACKNOWLEDGEMENTS

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Table 1: Live weight and feed intake.

	2x	6x	HB	HA	Contrasts
LW. change/28d, kg	10.7	8.3	12.0	7.5	-
Roughage intake, kg/d	8.1	8.3	8.8	8.8	2
Concentrate intake, kg/d	12.2	12.5	12.7	13.3	2
Total DM intake, kg/d	20.3	20.8	21.5	22.1	2

Contrast ($P<0.01$)
2 = No hay vs. hay

Table 2: Milk yield and composition.

	2x	6x	HB	HA	Contrasts
Milk, kg/d	28.6	28.7	29.7	30.1	2
FCM, kg/d	26.4	26.5	27.2	26.8	-
Fat, %	3.55	3.53	3.46	3.32	2
Protein, %	2.96	3.00	3.03	3.00	-
Lactose, %	4.92	4.98	4.98	5.00	1
Fat yield, kg/d	.99	1.00	1.01	.99	-
Protein yield, kg/d	.84	.85	.89	.89	2
Lactose yield, kg/d	1.41	1.43	1.49	1.51	2

Contrasts ($P<0.05$)
1 = 2x vs. 6x
2 = No hay vs. hay

Table 3: Rumen measurements.

	2x	6x	HB	HA	Contrasts
Rumen NH_3 - N, mg/dl	20.3	22.0	21.8	22.4	-
Rumen pH	6.35	6.20	6.18	6.15	-
Acetate, mol %	68.16	67.16	65.76	66.75	2
Propionate, mol %	17.36	17.08	18.25	18.00	-
Isobutyrate, mol %	1.54	1.58	1.22	1.36	2,3
Butyrate, mol %	9.97	10.98	11.33	11.00	2
Isovalerate, mol %	1.52	1.67	1.22	1.32	2
Valerate, mol %	1.46	1.52	2.23	1.56	2,3

Contrasts
2 = hay vs. no hay
3 = HB vs. HA

CLONING OF GENES CODING FOR D-XYLOSE ISOMERASE IN THE RUMEN BACTERIUM BACTEROIDES RUMINICOLA

D.J. Muysson, A.M. Gibbins and C.W. Forsberg[1],
Department of Animal and Poultry Science and [1]Department of Microbiology, University of Guelph.

SUMMARY

The rumen bacterium Bacteroides ruminicola is capable of metabolizing D-xylose, a major constituent of the hemicellulose fraction of forages. The objective of this research was to clone genes encoding enzymes involved in uptake and initial metabolism of D-xylose in B. ruminicola. The genes would then be available for the genetic manipulation of a predominantly cellulolytic rumen bacterium, B. succinogenes, which is unable to use D-xylose as a carbon source because of a lack of enzymes involved in xylose metabolism.

A genomic library (8500 clones; up to 9 kbp insert size) of B. ruminicola was created in the bacteriophage vector, λZAP. This vector was chosen since repeated attempts to create a genomic library using the plasmids pTZ18R and pBR322 failed, due to the inability to transfer B. ruminicola DNA to an Escherichia coli host in the plasmid form. Several strategies for cloning B. ruminicola DNA in plasmid form were attempted, including the use of a variety of restriction endonuclease combinations for the production of complementary sticky ends on genomic and plasmid DNA, and a variety of E. coli host strains (DH5α, HB101, K802, LE392, NM522, TG-1 and XL1-blue). However, none were successful.

The recombinant pBluescript DNA sequences were excised from the λZAP phage library, generating a library of plasmids initially packaged in phage coats (phagemids). The phagemid library was screened for the presence of a D-xylose isomerase gene by complementation of transfected E. coli SP10, a D-xylose isomerase-negative mutant. Four clones, SP10-7, SP10-4, SP10-3, and SP10-2 (insert sizes 275 bp, 6, 10, and 6 kbp, respectively) showed D-xylose isomerase activity at similar levels to that detected in B. ruminicola (cysteine-carbazole assay). Two of these clones (SP10-7 and SP10-2) will be analyzed further; SP10-4 and SP10-3 proved to be unstable. Attempts to transform E. coli SP10 cells with plasmid DNA isolated from the four E. coli SP10 clones failed.

The problems encountered in cloning B. ruminicola DNA in plasmid form but not in bacteriophage form might be attributed to the route by which the DNA is introduced into E. coli cells. Coated bacteriophage or phagemid DNA might be delivered to a subcellular compartment where replication and transcription of DNA can take place without degradation, thereby eluding cellular components which normally degrade naked plasmid DNA.

A more detailed account of the rationale for this project is given in the Guelph Dairy Research Report, 1988, pp. 202-210.

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RATE OF GROWTH AND MAMMARY DEVELOPMENT OF DAIRY HEIFERS IN TWO PERIODS PRIOR TO PUBERTY

J. Niezen and D.G. Grieve
Department of Animal and Poultry Science, University of Guelph

SUMMARY

In each of two trials, 20 Holstein calves were fed to grow at rates of 700 (L1) or more than 1,000 (H1)/d up to 200 kg liveweight. At 200 kg half of each treatment group was switched to the opposite treatment (L2, H2) until midway through the third estrous cycle. Four treatment combinations were thus L1L2, L1H2, H1L2, H1H2. Heifers from one trial are to be maintained for lactation experiments while those of the other were used to determine mammary development.

Age at puberty (first estrus) was advanced from about 11.5 mos. (L1L2) to 9.5 mos. (H1H2) by feeding for more rapid growth. Weight and wither height at puberty were not affected by treatment. Mammary glands of heifers on high plane of nutrition (especially H2) were heavier than those on low plane although proportions of fat and non-fat tissue were not affected by feeding level. Mammary DNA should be an estimator of cell density. In this experiment, amount of DNA was not affected, but concentration of DNA in fat-free tissue was higher for those heifers fed the low plane of nutrition (L1L2) than for those on high plane (H1H2). The data did not reveal any differential effect of plane of nutrition on mammary composition in the two periods (before or after 200 kg). It was therefore concluded that the critical period when feeding level affects mammary development extends over the whole post-weaning, prepubertal period.

INTRODUCTION

Previous studies have documented the effect that plane of nutrition during rearing exerts on mammary development and subsequent milk yield. Attempts to grow dairy heifers rapidly by offering a diet high in energy have resulted in reduced age at puberty, but in many instances also resulted in depressed milk production (Gardner et al, 1977; Little and Kay, 1979). Research at Guelph has attempted to define the levels of nutrition (or rates of gain) that will maximize reproductive development without impairing mammary development. In last year's report, it was shown that as growth rate of Holstein heifers was increased from 611 to 737 or 903 g/d between six and fifteen months of age, mammary glands contained decreased amounts of potential secretory tissue based on DNA analysis (Stelwagen and Grieve, 1988). A second goal of this research has been to develop means of determining mammary development in the live animal without interfering with long term performance. In the previous report, use of a mammary biopsy technique near puberty was found not to be effective for predicting mammary development (Stelwagen and Grieve 1988).

The objectives of the current research were firstly: to study effects of growth rate in two periods between weaning and puberty on mammary development in Holstein heifers; and secondly to evaluate ultrasonic scanning as a technique for predicting mammary composition.

PROCEDURE

In each of two parallel trials, 20 Holstein heifer calves were fed to gain 0.7 (L1) or > 1.0 (H1) kg per day until a liveweight of 200 kg was reached. Subsequently, half of the calves in each group was again fed for 0.7 (L2) or > 1.0 (H2) kg per day until 10 days after third estrus. There were thus four treatment combinations: L1L2, L1H2, H1L2 and H1H2. Appropriate weight gains were achieved by feeding differing quantities of the same diet comprised of alfalfa hay, grain corn, soybean meal and minerals - premix. The proportion of hay in the diet was increased between the first and second periods (at 200 kg). One group of animals is being retained for production information, while the second group was sacrificed at the end of the trial in order to determine mammary composition (dry matter, dry fat-free tissue (DFFT), crude protein, RNA and DNA). Puberty was determined from elevated plasma progesterone levels from weekly blood samples. All calves had mammary glands scanned ultrasonically at 200 kg liveweight and at 55 days past puberty (end of trial). Data from only the trial group sacrificed at the end of the trial are reported here.

RESULTS

Neither body weight nor wither height at puberty were affected by treatment (Table 1). Age at puberty however, was advanced by feeding for rapid growth. Those calves on high plane of nutrition throughout (H1H2) achieved puberty about two months earlier than those continuously on the low plane (L1 L2) with other treatments intermediate (Table 1).

Following slaughter midway through the third estrous cycle, mammary glands were recovered, trimmed of skin and teats and separated into left and right halves for further analysis. Glands from heifers on high plane of nutrition (especially after 200 kg liveweight) were heavier than from those on low plane although proportions of fat and non-fat tissue were not different (Table 2). Analysis of DNA and RNA content of mammary glands revealed no treatment effects except when expressed per unit mammary fat-free tissue (DFFT) (Table 3). In the latter case glands from heifers on high plane of nutrition throughout had lower concentrations of DNA and RNA than those on the low plane of nutrition.

Ultrasonic scans of mammary glands prior to slaughter were not closely related to mammary composition determined post slaughter. Correlation coefficients of ultrasonic measurement with DFFT were low and not statistically significant.

DISCUSSION

As expected, heifer calves raised on differing planes of nutrition reached puberty at similar body sizes. Average body weights of treatment groups at puberty in this experiment (274 to 295 kg) were similar to those reported previously (284 to 298) (Stelwagen and Grieve, 1988). Wither height at puberty in the present experiment was not affected by treatment. This suggests that growth in skeletal size was proportional to gain in weight and therefore body condition was not affected by rate of gain. This result is in contrast to that of last year's report where wither height at puberty decreased as feeding level increased indicating the rapid gains were due to fattening rather than proportional growth. Puberty was attained at about 9 months of age in calves on high plane of nutrition throughout (H1H2) compared to more than 11 months on those fed to gain at .7 kg per day throughout the trial (L1L2). These results lend support to the concept of puberty being more closely associated with body size than with age.

Analysis of mammary glands indicated that heifers fed the high plane of nutrition after 200 kg body weight had heavier glands but proportion of fat to DFFT was similar to that of heifers on low plane. This result is similar to our conclusion on body condition noted above but differs from previous work showing that increased gland weight was due to accumulation of fat.

Content of DNA in glands should be an estimator of cell density as each cell contains similar amounts of this material. In this experiment, total amounts of DNA were not affected by treatment but concentration of DNA in DFFT was higher for those fed the low plane of nutrition throughout than for those fed the high plane. This result is consistent with earlier reports. However, one of the objectives of this research was to determine relative importance of plane of nutrition or growth rate prior to versus after 200 kg liveweight (about 6 months of age). In these data effects from treatments in the two periods appeared nearly equal. Thus the critical period when plane of nutrition can affect growth of mammary parenchyma may encompass the total time at least from weaning up to puberty.

The results from use of ultrasonic measurements to predict mammary composition in prepuberal heifers were disappointing. Development of a simple yet effective method for measuring mammary development in the live animal would greatly assist research in this area.

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Table 1. Weight, height and age at puberty of Canadian Holstein heifers raised on one of four treatments from 118.4 kg until 55.5 days past puberty.

	Treatment*					Preplanned Contrasts*		
						Probability of Difference		
	H1:H2 (n=5)	H1:L2 (n=5)	L1:H2 (n=5)	L1:L12 (n=5)	SEM	H1 vs L1	H2 vs L2	H vs L
Weight (kg)	294.6	274.3	290.5	291.1	17.23	NS	NS	NS
Height (cm)	118.8	116.4	117.5	116.4	1.80	NS	NS	NS
Age (days)	278.8	310.4	306.4	343.8	16.38	NS	NS	.02

* H1 and H2 are defined as high plane of nutrition before and after 200 kg liveweight respectively.

L1 and L2 are defined as low plane of nutrition before and after 200 kg liveweight respectively.

H and L are defined as high and low plane of nutrition throughout the entire treatment period.

Table 2. Mammary gland components of Canadian Holstein heifers raised on one of four treatments from 118.4 kg until 55.5 days past puberty.

	Treatment*					Preplanned Contrasts*		
						Probability of Difference		
	H1:H2 (n=5)	H1:L2 (n=5)	L1:H2 (n=5)	L1:L2 (n=5)	SEM	H1 vs L1	H2 vs L2	H vs L
Trimmed Right Dry Wt. (kg)	1.56	.77	1.06	.78	.130	NS	.01	.01
Fat (%)	95.7	93.1	93.8	93.7	.678	NS	NS	NS
Dry Fat-Free Tissue (DFFT) (%)	4.28	6.93	6.23	6.30	.678	NS	NS	NS
Weight of Fat (gm)	1495.3	718.0	992.8	729.30	126.5	NS	.01	.01
Weight of DFFT (gm)	66.1	48.3	64.3	47.72	4.77	NS	.01	.02
Weight DDFT (mg/kg EBW)	292.7	260.7	294.3	248.9	14.80	NS	.02	NS

* H1 and H2 are defined as high plane of nutrition before and after 200 kg liveweight respectively.

L1 and L2 are defined as low plane of nutrition before and after 200 kg liveweight respectively.

H and L are defined as high and low plane of nutrition throughout the entire treatment period.

EBW = Empty Body Weight

Table 3. Mammary DNA and RNA expressed as total weight, per kg empty body weight (EBW) and per gram mammary fat free tissue (DFFT).

	Treatment*					Preplanned Contrasts*		
						Probability of Difference		
	H1:H2 (n=5)	H1:L2 (n=5)	L1:H2 (n=5)	L1:L2 (n=5)	SEM	H1 vs L1	H2 vs L2	H vs L
Total Weight								
DNA (mg)	560	490	660	519	60	NS	NS	NS
RNA (mg)	409	406	555	467	55	NS	NS	NS
RNA:DNA (mg/mg)	.74	.82	.85	.88	.06	NS	NS	NS
Weight/kg EBW								
DNA (mg)	2.49	2.66	3.05	2.71	.26	NS	NS	NS
RNA (mg)	1.82	2.21	2.56	2.44	.27	NS	NS	NS
Weight/g DFFT								
DNA (mg)	8.5	10.2	10.3	11.0	.66	NS	NS	.02
RNA (mg)	6.2	8.4	8.7	9.7	.76	.03	NS	.01

* H1 and H2 represent high plane of nutrition before and after 200 kg liveweight.

L1 and L2 represent low plane of nutrition before and after 200 kg liveweight.

H and L are defined as high and low plane of nutrition throughout the entire treatment period.

EXTENDING THE USE OF EMBRYO TRANSFER IN FARM ANIMALS

K.J. Betteridge[1] and C. Smith[2]
[1]Department of Biomedical Sciences and [2]Department of Animal and Poultry Science, University of Guelph

INTRODUCTION

Embryo transfer (ET) and related techniques are best considered as tools for improving the efficiency of livestock production rather than as ends in themselves. The efficacy of ET as a tool, and the applications for which it could be used in livestock production, were previously discussed at the 8th International Congress at Krakow in 1976 (1). The present paper will comment on some developments of ET during the intervening twelve years. There have been many reviews of the subject during this period (2 to 7) but it is hoped that further mention of some strengths and weaknesses of ET will extend its use, and encourage the solution of remaining problems. As in Krakow, attention will be focussed on cattle and divided between the techniques themselves, and their application.

TECHNIQUES

Production of embryos by superovulation

Approaches to superovulation have remained largely empirical. Despite claims and counterclaims about the superiority of FSH:LH (in various ratios) over eCG (with or without an antiserum to eliminate its residual effect after estrus), it remains impossible to predict accurately how an individual donor cow will respond to any particular treatment. Nevertheless, several important reviews and discussions of superovulation have been published since 1976 (e.g. Gordon in 3; 8,9) and a great deal of fundamental knowledge of normal folliculogenesis in sheep (10,11), cattle (8,9,12,13), horses (10,14) and pigs (15) has been obtained. In cattle, ultrasonographic studies (13) have shown follicular growth to occur in waves (usually three per cycle), as was hypothesized in principle nearly 30 years ago (16). During each wave, one follicle becomes dominant while the others regress. Superovulation depends on the subversion of the mechanisms underlying this selection of a single dominant follicle (13). Therefore, further detailed investigation of the effects of various treatment regimens on follicular growth and hormone production, oocyte maturation and ovulation are necessary if the yield of fertilizable oocytes is to be made more uniform. Measurable endpoints that can be associated with oocyte quality include steroid concentrations in follicular fluid (17), and the proteins and polypeptides produced by the oocytes themselves (18). Bovine FSH produced by recombinant DNA technology shows signs of being an improved superovulatory agent in cattle (19).

Production of embryos by *in vitro* fertilization (IVF)

As discussed more fully in the symposium on fertilization at this Congress, significant progress has recently been made in obtaining viable cattle embryos by IVF of oocytes taken from the ovaries of slaughtered cattle and then matured *in vitro* (20 to 24), even without the use of an intermediate recipient's oviduct for culture (22). Although this development had been foreseen in 1976 because of previous successful work with sheep (Gordon, in 1), it has been a long time materializing and the first IVF calf was not born until 1981 (25). Laparoscopic methods of oocyte collection should also allow IVF of *in vitro* matured oocytes to be used on selected donors (26,27).

Collection procedures

The transition from surgical to transcervical collection at Days 6-8 in cattle occurred quite abruptly in 1976 (28; Newcomb, in 3). Filtration of the uterine lavage has since been widely adopted to reduce the volume that needs to be searched to locate the embryos. Several filtration systems are commercially available. Although recovery rates cannot

be accurately gauged because of the difficulty of counting corpora lutea by palpation per rectum, it seems that collection procedures are quite effective.

Handling embryos *in vitro*

Circumstantial evidence suggests that many embryos may be lost as a consequence of *in vitro* procedures. For example, a 60 per cent pregnancy rate is generally regarded as "acceptable" and yet it means that 40 per cent of morphologically comparable embryos fail to develop to term (29). Although cytogenetic abnormalities do occur in normal-looking embryos at the time of transfer (30), their incidence is low (31). Consequently, much of the blame for the 40 per cent failure rate is often laid on the recipient. However, the success of ET in some species (e.g. the hamster) is extremely vulnerable to adverse conditions *in vitro* , including exposure of the embryo to daylight (32). In cattle, the length of time (beyond a few hours) for which embryos are held *in vitro* is inversely related to pregnancy rates (29), and the conditions under which embryos are held between collection and transfer are among the most inconsistent parts of current procedures. This is important because there may well be a considerable delay between the time when an environmental insult is inflicted upon an embryo, and the time at which the effect of the insult is manifested (29,33). This may explain why it is relatively easy to obtain cleavage following IVF, but much more difficult to achieve further development (34). Experimentally based recommendations on how best to handle embryos *in vitro* might go a long way towards maximizing the use of ET by reducing embryo wastage.

Embryo viability tests

Although the ultimate test of viability must always remain the production of healthy offspring, an objective measure of potential viability would be extremely useful. The exclusion, retention, or metabolism of various dyes have been proposed for this purpose, but morphological criteria remain the only ones widely used (33). Others are under investigation, including more elaborate morphological assessment using image analysis, the measurement of energy metabolism by individual embryos (35), and their production of materials such as platelet activating factor, various immunosuppressive agents and heat-shock proteins (33). While these investigations may be of limited direct application in the field, they could help expand the use of ET by helping the improvement of embryo handling.

Embryo sexing

Considering that the first sexing of bovine embryos was reported at the Krakow congress, progress in this area has been slow. Both the need for embryo sexing (36) and the price that could be paid for a reliable procedure (37) have been examined. Chromosome analysis applied to half-embryos (38) is reliable but takes time and is technically demanding. Its applicability is also limited by the difficulty of cryopreserving the remaining half-embryo. Immunological detection of male-specific antigens (39, 40) is reasonably reliable in skilled hands but still very subjective. Biochemical detection of X-linked enzymes before X-inactivation (41) has been used successfully in mice but not in other species. Detection of the Y-chromosome by species-specific DNA probes in a few cells holds great promise (42-44).

Micromanipulation of embryos

The production of multiplets by blastomere separation and nuclear transplantation was foreseen at the Krakow congress (Gordon, in 1). However, the main reason for the rapid passage of micromanipulative techniques from the laboratory to the ET industry (45 to 48) has been the relative ease with which late morulae and blastocysts can be divided into viable half-embryos. Nevertheless, blastomere separation is still useful; up to five identical offspring have been produced in sheep by separating the blastomeres of 8-cell embryos and combining each one with a blastomere from a 4-cell embryo (45). Similar manipulations have been

used to produce interspecies chimaeras between sheep and goats. By arranging for the trophoblast to match the recipient, goats have given birth to lambs and vice versa (45). However, the potential of this method for producing identical individuals may be surpassed by the technique of nuclear transplantation in which each separated blastomere is fused with an enucleated, activated oocyte (49, 50). The resulting embryos, each containing an identical nucleus, can then be cultured until they contain 8 to 32 cells. At this stage, the blastomeres can be separated to repeat the process, thus producing "clones" of embryos containing genetically identical nuclei. In evaluating the potential applications of these techniques of multiplet production, it is important to remember that the clones are of an embryo, not of either parent. In the case of nuclear transplantation, possible cytoplasmic effects on the 'genetic identity' of the clone will also need to be evaluated (50).

Chimaeras can also be produced by micromanipulation of post-compaction embryos by combining the inner-cell mass (ICM) of one with the trophoblast and/or the ICM of another. The significance of this will probably increase as the use of transgenic techniques extends, because, in mice, embryonic stem cells rendered transgenic *in vitro* have been incorporated into the germ line by injection into blastocysts (51).

Transgenic livestock production by microinjection of pronuclear fertilized oocytes will not be dealt with here, beyond stating the obvious fact that efficient gamete and embryo handling techniques, IVF, embryo culture and transfer are indispensable to this technology (6, 46, 47).

Disease control through embryo transfer

The potential for using ET to control disease transmission was discussed at Krakow (Mitchell & Betteridge, in 1). Techniques for realising this potential have become firmly established as a result of the considerable volume of experimental work since (52, 53). Consequently, defined procedures for washing embryos and monitoring media have been recommended to governments and agencies controlling the international movement of livestock. However, the acceptance of these recommendations still varies from country to country (52, 53).

Cryopreservation of embryos

At the time of the Krakow congress, only about 250 cattle embryos had been frozen worldwide and success rates were hardly known (Whittingham, in 1). Today, embryo cryopreservation is routine in commercial practice (54). Embryo freezing involves cooling at controlled rates in the presence of cryoprotectants to allow for dehydration as the temperature falls, thereby avoiding the formation of destructive intracellular ice crystals. Protocols for the procedure have been markedly simplified in recent years, as have procedures for rapid thawing and removal of cryoprotectant in hypertonic sucrose solutions before the embryo is rehydrated (Leibo, in 6; 5 to 57). Recently, an alternative method of cryopreservation by vitrification has been successfully used with cattle embryos (58).

Transfer procedures

Transcervical transfers were still in their infancy in 1976 (Brand & Drost, in 1) but have been used universally for several years now. Although this step in the procedure is not a serious limitation to ET in experienced hands (28; Newcomb in 3), many commercial users still obtain more consistent results with surgical transfer.

Criteria for selecting suitable recipients have been extensively investigated in commercial organizations and confirm the necessity of good synchrony between the donor, recipient, and stage of embryonic development (59). The synchronization of estrous cycles with prostaglandins and/or progestagens has simplified recipient management but should not be looked upon as an alternative to

careful observation and recording of behavioural estrus which remain central to the achievement of good results. Furthermore, some progestagen treatments of recipients appear to result in poorer pregnancy rates than those obtained in recipients synchronized with prostaglandins (60).

In small ruminants, laparoscopy is being used to speed transfer (61, 62), and also to carry out intrauterine inseminations, which are essential for good fertilization rates in sheep.

Transfer of trophoblastic vesicles along with embryos is being used to improve pregnancy rates (63). Perhaps the trophoblastic product(s) responsible for this improvement will prove a useful therapeutic adjunct to transfer (64).

The price of simplification?

A repetitive theme of this brief summary of changes in ET techniques over the past 12 years is that of simplification. This is a welcome development, but one that also has its dangers. When techniques are complex, strict attention to detail is necessary to achieve good results; as they become simpler, it is easy to let minor procedural details slip. Inattention to details as diverse as medium preparation, asepsis, embryo handling and estrus detection can be accumulative, and may explain why today's much more routine methods can still sometimes produce disappointing results.

APPLICATIONS

The extent of embryo transfer

An indirect measure of the growth and fluctuation of interest in ET since the Krakow congress is shown in Fig. 1. The expansion of its use in the predominant dairy breed in Canada is illustrated in Fig. 2. The Soviet Union and the countries of Eastern Europe are also making increasing use of ET, as is Cuba, where great emphasis is put on dairy products as a source of protein. The spread of ET in developing countries may depend on its wider use in small ruminants and buffalo (7). ET is also being used in non-traditional agricultural species such as deer (65).

Using embryo transfer for the genetic improvement of livestock

The ways in which ET can be used for this purpose have been recently reviewed (66). In dairy cattle, ET is now widely used for contract matings of bull mothers; 52 per cent of 1545 dairy bulls currently standing in six of Canada's major AI centres were obtained in this way. Using ET in contract matings increases the chances of getting useful bull calves, but has little effect on the rate of genetic change (67). However, ET can bring about useful gains in selection response by reducing the generation interval in progeny testing schemes. This may be achieved by using ET on young females, selected either on parental merit (68) or after their first lactation (69). Higher rates of genetic gain can be obtained within elite nucleus herds, bred by multiple ovulation and ET (MOET), and selected on records of full- and half-sisters instead of progeny (70). With current techniques, the rates of response in such herds are useful in adult schemes (ET after the first lactation) but are even higher when juveniles are used for ET before their first calf. In Europe, several such MOET-based breeding programmes are underway (66).

In beef cattle (71) and sheep (72), MOET has even more advantages over normal reproduction for improving rates of response, but practical adoption of the technique has been slow.

It is calculated that the costs of ET will have to be reduced by a factor of 5-10 before it can be economically justified for improving commerical, as distinct from nucleus, herds (37). It is an interesting paradox that sophisticated MOET schemes operating from centralized farms may be particularly useful in developing countries which lack the widespread recording systems that are essential to more

traditional methods of livestock improvement (66).

The growing use of ET for the international movement of livestock without risk of disease transmission is a less direct, but equally important, contribution to the exploitation of improved stocks in other countries.

Finally, the creation of transgenic livestock for improving livestock productivity in the future will depend on efficient gamete and embryo technology.

Embryo transfer as a direct tool of production

Cost considerations are obviously paramount in deciding whether ET is economically feasible for direct production purposes, such as increasing meat production by using beef breed embryos in dairy herds (Sreenan, in 1). The production of cheap embryos by IVF of oocytes collected from beef heifers at slaughter should bring this application a step closer.

Sexing embryos (or the destruction of male embryos) could have a direct application in efficient beef production in the "Single-sex, bred-heifer" scheme (73). Paradoxically, the use of female embryos would be required so that dams could be slaughtered early and replaced by their female calves.

Embryo transfer as a research tool

As was emphasized at Krakow (1) the very existence of an ET industry is the result of the reciprocal relationship between so-called "basic" research and its practical application. A specific example of reciprocity in this wide field of application for ET is research into the establishment of pregnancy and the causes of embryonic mortality. ET technology is indispensable to the study of the pre-attachment embryo and its environment; the studies it makes possible offer the most promise of reducing embryonic loss and increasing the efficiency of ET as a tool for livestock production (33). The provision of identical twins to make experimental groups will be very useful in many fields of investigation (74).

CONCLUSIONS

ET in farm animals has come of age as a useful tool for many aspects of livestock production. The radical simplification of the procedure may be a two-edged sword; while it has undoubtedly widened the span of applications, it may also have sometimes led to inattention to detail and an acceptance of success rates that could really be improved.

Superovulation is the weakest link in ET in cattle and seems likely to remain a limiting factor when a particular donor is to be used unless the bovine FSH produced by recombinant DNA technology lives up to its early promise. However, recent progress in IVF may help circumvent this problem, especially when the origin of the embryo is of less importance.

Remarkable strides have been made in embryo micromanipulation but there is much room for improvement in current methods of embryo sexing and viability testing. Similarly, although cryopreservation methods have developed very well over the past decade, they are still not ideal for conserving manipulated embryos.

The strict procedures for washing and handling embryos that have been recommended as a result of research into disease control by ET need to be carefully considered by governments and international agencies. Their acceptance should make the international movement of livestock a much easier and safer procedure than it is at present.

It is most encouraging that animal breeding strategies have been evolved to make the best possible use of ET as it now exists and to take advantage of technical improvements as they become available. As molecular biology provides yet more genetic tools for livestock improvement, the prospects for progress through the integration of diverse disciplines are very

promising. However, it must be remembered that today's applied technologies depend on work that could not have been justified on "practical" grounds when it was undertaken. As Mulnard has said of embryological research in human medicine, ". . . 'disinterested research' remains today, as ever, the surest warrant of new discoveries and of tomorrow's technological progress" (75).

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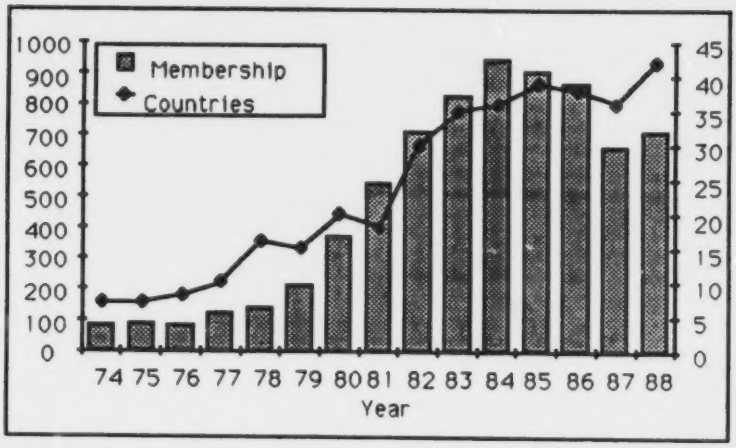

Figure 1. Membership, and countries represented, in the International Embryo Transfer Society since 1974.

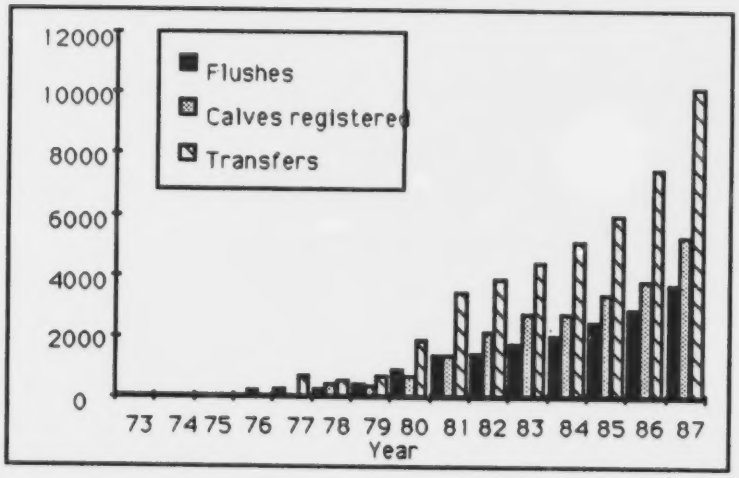

Figure 2. Embryo transfer activity in the Canadian Holstein breed in Canada since the first two calves were registered in 1973.

PERIPARTURIENT BEHAVIOUR OF DAIRY COWS AND NEONATAL BEHAVIOUR OF CALVES

H. Houwing, J.F. Hurnik and N.J. Lewis
Department of Animal and Poultry Science, University of Guelph

SUMMARY

The preparturient behaviour of 16 Canadian Holstein cows and heifers, the postparturient behaviour of 31 Canadian Holstein cows and heifers and the postnatal behaviour of 32 calves was analyzed. Behavioural observations of dams started 12 hours prior to delivery. This period was divided into 4 intervals of 3 hours. The postpartum period consisted of 2 intervals, the first 3 hours after delivery and the fourth hour until separation of the calves from their dams. After separation, the dams were observed until 24 hours postpartum.

In the 12 hour interval prior to parturition, there was a gradual increase in time spent in semi-lateral recumbency, a decrease in standing and ruminating time, and also a decrease in the length of individual standing and ruminating events. Two cows (13%) and five heifers (31%) were observed to be standing during the birth of their calves, while 13 cows (87%) and 11 heifers (69%) were recumbent. Delivery without assistance occurred in 10 of 32 cases (31%), 8 calves (25%) were born with little assistance, 12 (37%) with the use of delivery tools and 2 calves (6%) required veterinary assistance. When no assistance was required, only one animal was standing during the birth of the calf. The 9 dams which were recumbent during parturition and required no assistance were standing sooner after parturition than those in a recumbent position with assistance (4 minutes vs 37 minutes, respectively). After delivery, heifers required a longer time to stand up than cows (37 minutes vs 14 minutes, respectively).

Several significant differences were observed in the behaviour of neonatal calves. First standing, first suckling attempt and the commencement of successful suckling were performed sooner by female calves and calves born to heifers. These differences could be partially explained by the lower body weights of female calves and calves born to heifers. The average length of the individual suckling events during the first 3 hours of life was 11 minutes. After removal of the calves, there was a significant increase in the amount of time spent in lateral recumbency by the dam, which may represent a period of physical recovery. The dams also spent more time ruminating, eating and self-licking in comparison to the period prior to separation of the calf.

INTRODUCTION

Reliable knowledge of behavioural activities of farm animals is essential for good management practice and proper housing. There have been several investigations into the behaviour of cows and calves during the periparturient period (Hafez and Lineweaver, 1968; Selman et al., 1970; Lewandrowski and Hurnik, 1983; and Metz, 1985). Nevertheless, there is still a distinct need to study this dynamic period of animal life in order to minimize losses and improve handling routines of dams and calves. The main objective of this experiment was to study the behaviour of primiparous and multiparous dams and to discern differences between male and female calves, using continuous video monitoring, eliminating the potentially interfering presence of the observer in the maternity pen.

PROCEDURE

Fifteen multiparous (cows) and sixteen primiparous (heifers) Canadian Holstein dams from the Elora Dairy Research Centre were observed continuously in the periparturient period, commencing 12 hours before and ending 24 hours after parturition. The total observation time was divided into 3 periods (A, B, C):

- prepartum period (A) was divided into 4 consecutive intervals of 3 hours;
- postpartum period with calves (B) was divided in the first 3 hours (Ba) and fourth to separation (Bb);
- postpartum period (C) until 24 hours post-separation.

The cows and heifers gave birth to 20 male calves and 12 female calves (one cow delivered twins).

For each prepartum period, the average percentage of time spent in a given behavioural state and the duration of each behavioural event (defined in Hurnik et al., 1985) were calculated. Only cases (16) in which the full 12 hours were available were analyzed. All other analyses utilized the full sample of 31 dams. The exact intervals between birth and the first occurrences of head lifting, attempts to stand (forelegs first), attempts to stand (hindlegs first), standing, attempts to suckle and suckling were registered for each calf from the video tapes. At five minute intervals, observations were made of the following mutually exclusive behavioural states of the dam and the calf: lateral recumbency (lying with lateral ground-contact of the body, head and legs), semi-lateral recumbency (lying with lateral ground-contact of the posterior part of the body and sternal contact of the thorax), standing and walking. In addition, the following behavioural activities for each dam were also recorded: eating, drinking, ruminating, self-licking, sniffing and licking the calf's body, licking the ano-genital area of the calf and eating the placenta.

The dams and their calves were housed in calving pens (2.70m X 3.70m) with a concrete floor covered with rubber mats and straw. Following the routine practice of the station, pregnant cows and heifers were moved to a calving pen 2 to 4 days before the expected delivery and the calves stayed with their dams for a minimum of 5 to a maximum of 17 hours after delivery. Daily cleaning of the maternity pens took place at 1000h, milking occurred at 700h and 1700h and feeding at 500h and 1300h. Feed and water were available ad libitum. The intensity of illumination (65 lux) was constant, allowing 24 hour/day video recording with a low light-intensity camera and a time-lapse video-cassette recorder.

Statistical contrasts were used to detect linear and quadratic effects of time on the recorded activities. The differences between the individual periods were analyzed using t-tests. The Mann-Whitney U-test (Siegel, 1956) was used to analyze the elapsed time to first occurrence of behavioural activities for female versus male calves, and those born to cows versus heifers. The same test was used to assess the differences in the percentage of time calves born to primiparous and multiparous dams spent in these activities. The Spearman Rank-correlation coefficient (Siegel, 1956) was calculated to assess the relationship between the weight of the calves and the time of the first occurrence of a behavioural activity. Statistical significance for all tests was accepted at the $P<0.05$ level.

RESULTS

Prepartum Activities

In the 12 hours prior to delivery, the percentage of time spent standing increased marginally, then dropped in the 3h prior to delivery (Figure 1). Lateral recumbency declined from period 1 (-12 to -9h) to periods 2 and 3 (-9 to -3) then rose in the final 3h before delivery (Figure 1). Semi-lateral recumbency showed a slight decline in the percentage of time through -12h to -3h and then a rapid increase in the last 3h (Figure 1). Rumination time gradually declined over the recorded period (Figure 1), starting 9h before delivery. The other observed behaviours did not significantly vary with approaching parturition.

The average duration of individual behavioural events during the prepartum period were also affected by impending parturition. The duration of standing decreased gradually from period 1 to period 3 (-12h to -3h), then dropped rapidly during the final 3 hours before parturition (Figure 2). The average duration of individual rumination events started to decline 6 hours prior to delivery (Figure 2). Recumbency events showed some changes in duration, but these did not appear to be significantly related to approaching parturition.

Two cows (13%) and 5 heifers (31%) were observed to be standing during the birth of their calves, while 13 cows (87%) and 11 heifers (69%) were recumbent. No assistance was required in 10 of 32 cases (31%), 8 calves (25%) were born following simple manual assistance, 12 (37%) with the use of delivery tools and 2 calves (6%) required veterinary assistance. When no assistance was required,

only 1 animal was standing during the birth of the calf. The 9 dams which were recumbent during parturition and required no assistance were standing sooner after parturition than those in a recumbent position with assistance (3.6, s.d. $\pm$ 2.6 min and 37.3, s.d. $\pm$ 34.2 min respectively; $P<0.05$). Multiparous dams stood sooner after delivery (14.4, s.d. $\pm$ 22.9 min) than heifers (36.8, s.d. $\pm$ 44.6 min).

Latency Between Birth and the First Behavioural Activities

The elapsed time between birth and the first occurrences of the recorded post-natal activities were generally shorter for the female calves and significant differences between sexes existed for the time to the first attempt to stand by stretching the hindlegs, the first attempt to suckle, and the first suckle (Table 1). The initiation of some activities of the calves were also influenced by parity. Calves from heifers attempted to suckle their dam and suckled sooner than calves from cows. Calves from heifers also required fewer standing attempts than calves from cows (Table 1). An attempt to suckle within the first six hours after birth was observed in only 8 (50%) of the cows' calves, while all of the heifers' calves made distinct attempts to suckle within the same period. Ten (62.5%) of the cows' calves, but only 1 (6.3%) of the heifers' calves were not observed to suckle within 6 hours of birth.

Prepartum Versus Postpartum Behaviours

Comparisons between prepartum and postpartum periods show a difference in the average percentage of time spent standing, walking and ruminating (Table 2, Contrast A-B). Standing increased from 49% to 58% of the total time while walking and ruminating decreased from 3% to 1% and from 18% to 7%, respectively.

Postpartum Behaviour

The behaviour of the cows and calves changed between the two defined postpartum periods in which the dam and calf were together (Table 1, Contrast Ba vs Bb). Licking the calf's body decreased from an average of 36% of the time in the first 3 hours to an average of 10% after the fourth hour. Licking the ano-genital area decreased from 7% to 3% of the time. In the second period, starting 3 hours after delivery, the dams appeared to be returning to prepartum levels for lateral and semi-lateral recumbency, and standing. Eating, ruminating and licking the cows' own body occupied more time in the second postpartum period and the same trend was visible when the periods before and after dam-calf separation were compared (Table 1, Contrast B vs C). In the period after separation, the time spent in lateral recumbency was also longer than that observed prior to separation.

The length of individual behavioural events in the periparturient periods are listed in Table 3. Periods of lateral recumbency and standing were shorter before parturition than after parturition, walking and ruminating events were longer. Differences in the lengths of individual behavioural events in the periods were often similar to the average percentage of time spent in various activities, i.e. a longer average duration of the event resulted in a higher percentage of time spent in a given activity. However, the average duration of lateral recumbency events before parturition was shorter than the duration of events between parturition and separation, while the average percentage of time spent in lateral recumbency before parturition was longer. A similar discrepancy was observed for the behaviour of licking the cow's own body in the two periods between parturition and separation. In the first 3 postpartum hours the time spent self-licking was less than 1% and in the second period (fourth hour to separation) close to 3%, but the average duration of an individual self-licking event was longer in the first postpartum period. This may indicate a reduced activation threshold for self-licking immediately after delivery, but once such an activity was initiated, positive feed back extends the performance of this activity over longer periods of time.

Early Postpartum Versus Later Postpartum Behaviour

Differences in behaviour were also noted (Table 3) between the first 3 hours after parturition (Ba), and 4 hours after parturition to separation (Bb). The average event duration of the dam's standing, drinking, self-licking, licking the calf's body and licking the ano-genital area

of the calf, were all longer in the first 3 hours. The average event durations of lateral recumbency, eating and ruminating were significantly shorter. After removal of the calf, eating, ruminating and self-licking events became longer (Table 3, Contrast B vs C).

The average percentages of the calves' time which was spent performing individual behaviours in the two periods between parturition and separation are given in Table 4. Less time was spent in lateral recumbency and more time in semi-lateral recumbency in the first 3 hours after delivery. As may be expected the numbers of attempts to stand was also higher in the first 3 hours of calves' lives. Table 5 shows the differences in the time spent performing individual behaviours for the calves born to cows and heifers. Prior to separation from their dams calves from heifers walked and suckled longer than calves from cows. Calves from heifers attempted to suckle more in the first 3 hours, while calves from cows tried to suckle more in the later period ($P<0.05$). A significant difference was also detected between male and female calves in the development of standing. Female calves spent more time standing in the first period, while male calves spent more time standing in the second period postpartum ($P<0.05$). The average duration of a single event was shorter for lateral recumbency and walking in the first 3 hours of life, while individual events of semi-lateral recumbency and standing were shorter (Table 6).

CONCLUSION

The observations collected in this experiment indicate distinct variability in periparturient behaviour of dairy cows and their calves. The most significant factors influencing the collected data were the age of the dam (primiparous vs multiparous dams) and the sex of the calves. This effect could be related to body weight differences, as the calves from the cows were heavier than those from the heifers and the male calves were heavier than the female calves. After removal of the calves there was a significant increase in the amount of time spent in lateral recumbency by the dam, which may indicate a period of recovery. More time was also spent ruminating, eating and self-licking after separation from the calf. This increase in time spent in ruminating and eating may be applicable to the predominance of maternal activities prior to separation of the calf, coupled with reduced activation thresholds for ruminating, eating or self-grooming in the presence of the calf. Licking of the cows own body may also be an indicator of redirected behaviour, generated through frustration by the separation from the calf. Nevertheless, the composition of behavioural activities of the dams after separation shows a relatively rapid return to early preparturition levels.

ACKNOWLEDGEMENT

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Table 1. Interval from Birth to the First Occurrences of Behavioural Activities.

	male		female		cows		heifers		all	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
Dams:										
standing (min)	22.2	22.0	28.1	50.1	14.4*	22.9	36.8*	44.6	24.7	35.6
licking calf (min)	2.7	4.2	2.6	3.0	2.6	4.8	2.7	2.5	2.7	3.8
Calves:										
head lifting (min)	3.8	5.3	1.9	1.2	2.5	2.8	3.6	5.5	3.0	4.2
standing attempt (min):										
- forelegs first	23.3	17.1	15.7	12.2	17.5	14.9	23.3	16.4	20.4	15.7
- hindlegs first	34.9*	19.2	23.3*	13.3	30.1	18.6	31.1	17.8	30.6	17.9
standing (min)	152.2	103.2	88.0	41.1	123.3	110.4	81.9	48.5	107.0	89.2
standing attempts (#)	17.6	10.1	15.4	9.0	20.6*	11.4	11.9*	4.0	16.4	9.6
sucking attempt (min)	1173.1*	75.3	90.5*	41.0	193.1**	72.5	110.2**	59.9	140.1	75.1
sucking (min)[1]	188.7*	90.3	99.3*	43.3	233.7**	70.4	112.6**	61.0	152.1	86.0

* Stars indicate a difference between male (n = 20) and female (n = 12) calves or between cows (n = 15) and heifers (n = 16): * = $P \leq 0.05$, ** = $P \leq 0.005$.

[1] Calves which required more than 6 hours were excluded from the average value (10 of the cow's calves and 1 of the heifer's calves).

Table 2. Average Percentage of Time Spent Performing Each Behaviour in the Distinguished Periods (A,B,C) for the Dams.

	N = 16			N = 31					
	%		P<	%				P ≤	
	A	B	A-B	Ba	Bb	B	C	Ba-Bb	B-C
lateral recumbency	7.1	4.6	NS	2.2	6.9	5.7	10.3	.005	.005
semi-lat. recumb.	40.7	36.6	NS	27.7	42.7	37.9	39.3	.05	NS
standing	49.4	57.6	.025	68.8	49.5	55.1	49.0	.005	NS
walking	2.7	1.2	.025	1.3	1.0	1.2	1.4	NS	NS
	99.9	100.0							
eating	7.7	7.1	NS	4.6	9.8	7.5	16.1	.025	.001
drinking	0.0	0.2	NS	1.3	0.1	0.5	0.4	.05	NS
sniffing,licking	2.2	1.3	NS	1.7	1.7	1.7	1.5	NS	NS
ruminating	18.1	6.9	.001	0.5	10.6	7.7	19.9	.001	.001
self-licking	1.4	1.5	NS	0.7	2.9	2.2	4.0	.001	.001
eating placenta	-	-	-	1.7	4.8		-	NS	-
licking calf's body	-	-	-	35.7	9.6		-	.001	-
licking ano-genital area	-	-	-	7.5	3.1		-	.001	-

Table 3. Length of Behavioural Events in Minutes in the Distinguished Periods (A,B,C) for the Dams.

	N = 16			N = 31					
	Min		P<	Min				P ≤	
	A	B	A-B	Ba	Bb	B	C	Ba-Bb	B-C
lateral recumbency	7.8	8.9	.05	8.0	8.8	8.7	8.7	.005	NS
semi-later.recumb.	17.2	16.6	NS	16.8	17.0	17.0	19.3	NS	NS
standing	24.4	27.4	.025	34.9	24.0	27.3	27.6	.05	NS
walking	6.3	5.3	.05	5.4	5.0	5.1	5.2	NS	NS
eating	7.4	6.7	NS	6.4	7.1	6.9	9.5	.005	.001
drinking	5.0	5.0	NS	6.0	5.0	5.9	5.0	.05	NS
sniffing,licking	5.6	5.8	NS	5.3	5.6	5.5	5.1	NS	NS
ruminating	17.4	15.6	.001	8.8	15.3	15.1	17.6	.001	.001
self-licking	5.7	5.5	NS	5.4	5.2	5.3	5.7	.001	.001
eating placenta	-	-	-	13.6	16.8	16.2	-	NS	-
licking calf's body	-	-	-	10.7	6.3	8.4	-	.001	-
licking ano-genital area	-	-	-	6.6	5.6	6.1	-	.05	-

Table 4: Average Percentage of Time Spent Performing each Behaviour in the Distinguished Periods (Ba Bb) for the Calves.

	%		P ≤
	Ba	Bb	Ba-Bb
lateral recumbency	14.2	41.7	.001
semi-later.recumb.	65.2	32.0	.001
standing	19.5	24.3	NS
walking	1.2	2.1	NS
attempt to stand	2.6	0.6	.025
attempt to suckle	1.1	1.7	NS
suckling	4.9	4.4	NS

Table 5. Average Percentage of Time Spent Performing each Behaviour for Calves from Multiparous (C) and Primiparous (H) Dams

	%		P ≤
	C	H	C-H
lateral recumbency	25.5	29.6	NS
semi-later.recumb.	53.5	44.7	NS
standing	20.1	23.5	NS
walking	0.8	2.2	.05
attempt to stand	1.9	1.0	NS
attempt to suckle	1.3	1.2	NS
suckling	1.4	7.1	.001

Table 6. Length of Behavioural Events in Minutes in the Distinguished Periods (Ba,Bb) for the Calves.

	min		P≤
	Ba	Bb	Ba-Bb
lateral recumbency	11.1	18.9	.001
semi-later.recumb.	27.3	11.1	.001
standing	14.9	14.0	.025
walking	5.0	5.5	.05

Figure 1. **Temporal Distribution of Preparturient Behaviour**

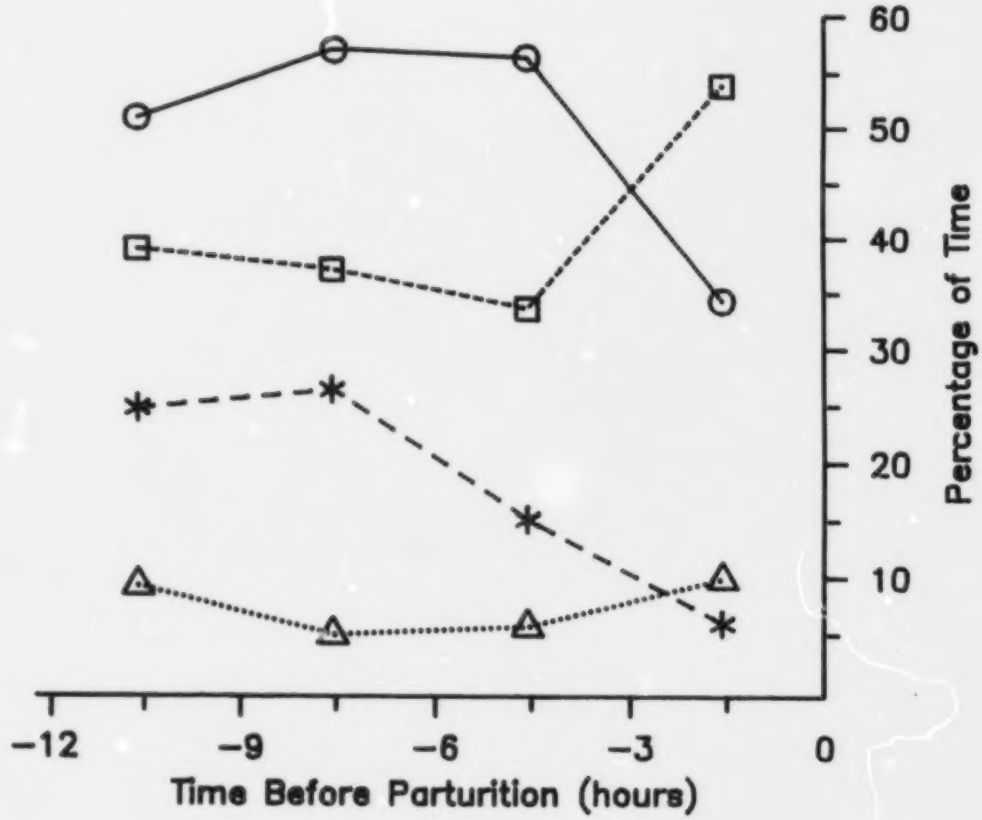
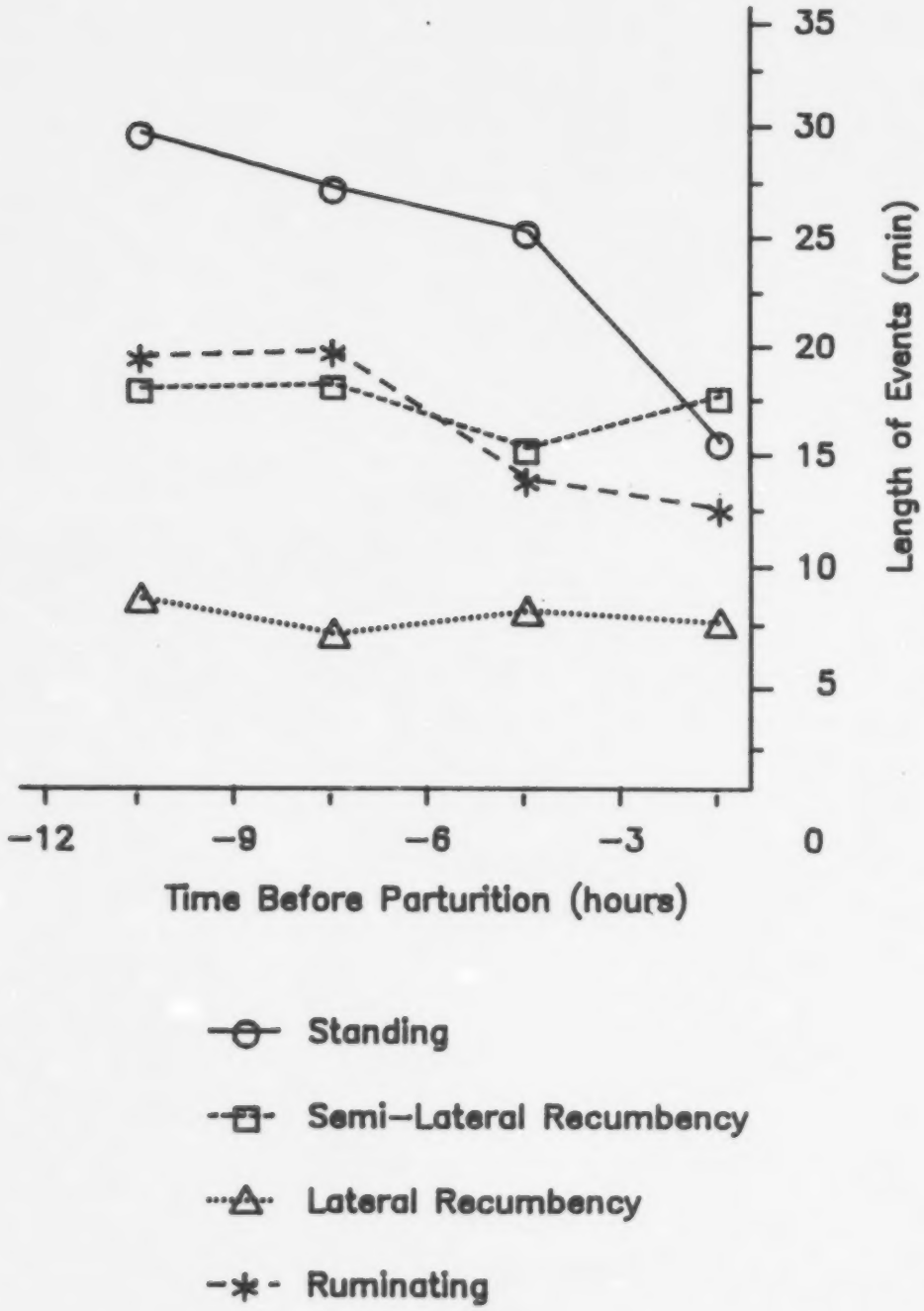


Figure 2. **Average Duration of Preparturient Behaviour**



LIVESTOCK EMBRYO SEXING: A REVIEW OF CURRENT METHODS, WITH EMPHASIS ON Y-SPECIFIC DNA PROBES*

R.A. van Vliet, A.M. Verrinder Gibbins and J.S. Walton
Department of Animal and Poultry Science, University of Guelph.

SUMMARY

Control of the sex ratio of domestic species is potentially of great commercial importance to agriculture. While sexing of spermatozoa would be the most advantageous approach, studies to date suggest that this technology is unlikely to be available in the near future.

As an alternative, four methods of sexing embryos have been developed. The use of X-linked enzymes and a serological assay involving H-Y antigen are noninvasive methods which have the advantage of allowing all embryos to be sexed, but these methods are not always accurate. Cytogenetic analysis and the use of Y-specific DNA probes are invasive methods which are limited by the accessibility of embryonic material for biopsy, but they are highly accurate. Each method is reviewed, with an emphasis on the use of Y-linked probes, and each is seen to have both advantages and limitations; the difficulty is in achieving a method that provides both an accurate sexing procedure and an acceptable pregnancy rate after embryo transfer. While no single method currently available fulfills all the criteria for a commercial method of embryo sexing, the potential for the development of an ideal method does exist.

INTRODUCTION

Preselection of the sex of offspring of agriculturally important species has long been an objective of animal breeders. However, not until the advent of technologies such as artificial insemination and embryo transfer has such an approach been considered commercially feasible.

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The ideal method of controlling the sex ratio would be to separate Y-and X-bearing spermatozoa, and to introduce the separated spermatozoa by pipet. These methods have been based largely on differing physical characteristics of X- and Y-bearing spermatozoa, such as differential mass (the X chromosome contains more DNA than the Y chromosome), volume, density, charge and motility, and include fractionation on albumin columns, Sephadex gel filtration, electrophoretic separation and various types of flow fractionation (1). To date, none of the methods developed has been generally accepted as successful. In a recent study, Johnson (2) evaluated the claims of many different academic and commercial institutions to have produced semen with a skewed sex ratio. Using flow cytometric analysis, which measures the relative DNA content of each cell (but is in itself a destructive process), Johnson determined from analyzing more than 200 samples that the spermatozoa sex ratio in each case was 50:50. Conceivably, the separation of X- and Y-bearing spermatozoa could be carried out by examining differences based on genetic expression. The difficulty with this is that the DNA stored in the head of the spermatozoon is highly condensed and bound to basic proteins, known as protamines, by the end of spermiogenesis. Genetic expression in the mature spermatozoon is limited until after penetration of the oocyte, thereby reducing the possibility of detecting sex-specific proteins in the interim. Y-specific DNA probes have been used to sex spermatozoa (3); however, since the spermatozoa must be destroyed to allow access of the probes to the DNA in the nucleus, this method is of little practical use.

The alternative approach to sex selection involves sexing embryos. Preliminary studies suggest that methods for sexing embryos do show potential for commercial use (4). Embryo sexing will be used in conjunction with embryo transfer and most of the research has been geared to sexing bovine and equine embryos, which can be transferred nonsurgically up to the blastocyst stage. However, methods for sexing porcine and ovine (5) or murine (5, 6) embryos as well as human fetuses (7-9) have also been developed. Sexing methods are classified as either invasive or noninvasive, depending on whether or not a biopsy of

embryonic tissue is required (10).

Criteria that must be considered in embryo sexing techniques are the percentage of embryos that can be accurately sexed and the effect that the sexing procedure may have on embryo viability. For example, a method which guarantees that 80% of embryos can be sexed, and that 60% of pregnancies from sexed embryos survive to term, has only a 48% probability of yielding accurately sexed offspring, a figure which differs little from the probability of male or female offspring occurring in the unmanipulated pregnancy. Thus, the difficulty in sexing embryos effectively lies in the necessity to achieve a method which provides both an accurate sexing procedure and an acceptable pregnancy rate after embryo transfer.

This review focuses on recent developments in the methodology of embryo sexing, with an emphasis on the use of Y-specific DNA probes. An evaluation of the potential of these methods for commercial use is considered.

BIOCHEMICAL AND IMMUNOLOGICAL METHODS OF EMBRYO SEXING

Noninvasive Methods

A noninvasive approach is generally considered optimal because it maintains the integrity of the embryo (10) and so is less likely to impair the potential for successful embryo transfer and implantation. Two methods fall into this category: the monitoring of X-linked enzyme activity prior to X chromosome inactivation, and the immunoreaction of embryos with antibodies to a sex-specific antigen.

X-linked Enzymes. In normal mammals the homogametic sex carries two X chromosomes, whereas the heterogametic sex possesses only one (an XX versus XY sex chromosome complement). To maintain an equivalent number of genes between the sexes, one of the X chromosomes of the female is inactivated in each cell early in embryonic life (11). Although the exact timing of X-inactivation is not known, studies have suggested that there is a brief period between activation of the embryonic genome and X-inactivation in which genes from both X chromosomes in the female are transcribed (12). This is reflected in a cellular concentration and activity of certain X-linked enzymes that is twice as high in female as in male embryos. In both cases, X-linked enzyme activity must be compared to autosomal-linked enzyme activity to account for individual variation in embryo metabolism.

Based on the hypothesis that the ratio of X-linked enzyme activity to autosomal enzyme activity will be higher in female than in male embryos, Williams (6) and Monk and Handyside (13) have sexed pre-implantation embryos from superovulated mice. In the study conducted by Williams (6), morula to blastocyst-stage embryos were examined for activity of the X-linked enzyme, glucose 6-phosphate dehydrogenase (G6PD). Briefly, embryos were incubated in a medium containing the substrate glucose 6-phosphate, the co-enzyme NADP and the indicator dye, Brilliant Cresyl Blue. Embryos were then scored on the intensity of the resulting stain (with the darkest and lightest stains indicating low and high G6PD activity, respectively) and transferred to surrogate mothers. Embryos fell into a bimodal distribution according to G6PD content, with embryos from the lightly stained categories (high G6PD activity) assumed to be female and those from the darkly stained categories (low G6PD activity) assumed to be male. Birth of the pups showed that 72% (62/86) of female embryos and 57% (54/95) of male embryos were correctly identified as to sex. The combined birth rate was 35% (181/516) compared with 46% (39/84) for controls. Embryo mortality was highest in the two extreme staining categories.

In the study by Monk and Handyside (13), single blastomeres were isolated from zona-free eight-cell mouse embryos and examined for X-linked hypoxanthine phosphoribosyl transferase (HPRT) activity. Autosome-linked adenine phosphoribosyl transferase (APRT) activity was also determined to control for the differing basal rates of cellular activity of the various embryos. Both HPRT activity and the ratio of HPRT:APRT activities yielded a bimodal distribution similar to that obtained by Williams (6) for G6PD activity. Following transfer of embryos to

pseudopregnant recipients, 21 sexed and 26 control embryos yielded 15 (71%) and 19 (73%) pups, respectively. Accuracy of sexing was 91% (11/12) for females and 100% (3/3) for males.

These studies raise several points of interest. First, the high mortality rates in the extreme staining categories determined by Williams (6) suggest that the assay procedure was somewhat toxic to the embryos, either because of extensive enzyme activity or retention of the stain (6). The alternative method proposed by Monk and Handyside (13) of removing a blastomere for sexing (thereby categorizing this method as invasive) involved eight-cell embryos, and has the limitation of being potentially difficult to adapt to compacted morula or blastocyst-stage embryos. Second, the exact period of X-inactivation in domestic species is not known (14). Thus in the experiments of Williams (6) and Monk and Handyside (13), the faulty diagnosis of female embryos as male by X-linked enzyme activity may have been due to early X-inactivation of some of the female embryos (6). Third, because activation of the embryonic genome may occur as late as the 8-cell stage in mice (15), and generally occurs between the 8- and 16-cell stage in cattle (16), care must be taken in such assays to ensure that the enzymes monitored are the result of embryonic transcription and not derived from the translation of stored maternal messenger RNA. Finally, as pointed out by Reiger (14), who has conducted experiments using G6PD to evaluate embryo viability, the level of activity of any enzyme is a function of total cellular activity. The necessary evaluation of cellular activity in this method also provides an index of embryo viability.

H-Y antigen. The immunological demonstration of a sex-specific antigen provides a second noninvasive method of sexing embryos. Studies in this regard have focused on the histocompatibility Y antigen (or H-Y antigen), which was first identified when females from an inbred strain of mice rejected skin grafts from their male litter mates although there was no rejection of female skin grafts in the males (17). Although the function of this antigen is not known, the gene has recently been mapped to the long arm of the Y chromosome (18), and has been proposed to be one of perhaps many genes involved in organizing the indifferent gonads into testes.

In 1971, Goldberg et al. (19) reported a serological assay for H-Y antigen involving antibodies isolated from the serum of females which had rejected male skin grafts; these antibodies were capable of identifying male cells in serological systems. Because the serological assay relies on a B-cell response, while the initial identification of H-Y antigen relied on a cytotoxic T-cell response, there has been some debate as to whether or not the two assays refer to the same antigen (20). The serologically isolated antigen (which will be referred to as H-Y antigen for simplicity) appears to be male-specific, however, and is the means by which embryos are sexed immunologically.

Two methods exist for detecting H-Y antigen on embryos: a cytotoxicity assay and an immunofluorescent assay. In the cytotoxicity assay, embryos are exposed to dilute H-Y antiserum and complement. Embryos expressing H-Y antigen show a degree of cell lysis, and thus are categorized as male (21). In an experiment in which 1000 8- to 16-cell mouse embryos were sexed by the cytotoxicity assay, 479 (48%) were classified as male (22). Following transfer of unaffected embryos to pseudopregnant recipients, 50 of 58 pups born (86%) were female. However, embryo survival in this study was low in both the experimental and control groups, a factor which appears to be due to the culturing of the embryos prior to transfer (22). Further, the cytotoxicity assay is marred by the necessary destruction of the male embryos.

The immunofluorescent assay system involves exposing embryos to primary (H-Y) antibody for 30 min., followed by reaction with a secondary antibody to which fluorescein isothiocyanate (FITC) has been conjugated. Embryos are then evaluated for the presence or absence of the FITC tag under a fluorescent microscope (21). In a recent experiment in which 258 four-cell to blastocyst-stage bovine embryos were subjected to the fluorescent immunoassay, 132 were classified as fluorescent, while 126 were nonfluorescent (23). When cytogenetic analysis was used to confirm the

sex of the embryos, of the presumed male embryos, 67 of the 84 (79%) readable karyotypes were XY. Of embryos classified as nonfluorescent, 58 of 65 (89%) were XX. Fertility trials of sexed embryos using this method are limited, but Wachtel reported (21) that in a preliminary trial using eight sexed bovine embryos seven survived to term (with only one of the calves having been typed incorrectly). However, an accurate determination of calving rates for sexed bovine embryos must depend on a larger field trial.

The major disadvantage of the immunological sexing assay is that the accuracy of sexing does not appear to exceed 87%, despite the development of new types of assays and the use of monoclonal rather than polyclonal antibodies (4). Several reasons have been proposed for this. First, because H-Y antigen is a relatively weak antigen, the antibodies generated may not be sufficiently specific, resulting in cross-reactivity of the antibody with other cell-surface antigens. Second, in the immunofluorescent assay, the secondary antibodies occasionally show nonspecific-binding, for example, to cellular debris in the perivitelline space (23). Third, H-Y antigen expression may not be limited exclusively to male embryos. For example, Wolf et al. (24) found that human Turner syndrome patients (45, XO) were serologically H-Y-positive, but at reduced levels. This again leads to the perplexing question of whether or not the serologically-identified antigen is equivalent to the transplantation antigen originally identified by Eichwald and Silmser (17) and localized on the long arm of the Y chromosome by Simpson et al. (18). Finally, the assay may be limited by the subjectivity involved in judging the degree of fluorescence of the antibody tag.

Efforts are currently being focused on the generation of new types of assays to identify H-Y antigen and on the use of this method to sex embryos of other mammalian species. To date, sexing rates of 81 to 85% have been obtained for porcine and ovine embryos (5, 25). Modifications of the assay procedure include the substitution of a reporter enzyme such as horseradish peroxidase in place of FITC (21) as well as the use of biotin-avidin to increase the amount of enzyme bound to the secondary antibody. Because enzyme immunoassays are easily packaged in kit form, the objective of these studies is to generate a test that can be carried out at the site of embryo transfer.

Invasive Methods

Cytogenetic analysis. Cytogenetic sexing, or karyotyping, was the first method to produce sexed rabbits, calves and sheep (10). Briefly, the technique involves the biopsy of a small number of cells which are cultured in a medium with colcemid, a mitosis-arresting agent (26). The cells are then induced to swell so that the chromosomes disperse. Following fixation and staining with a permanent DNA dye, such as Giemsa, the slides are examined under a microscope. Cells which were arrested in metaphase generate a spread of chromosomes which can be identified by their specific banding patterns. The Y chromosome is easily identified by its small size and, in the case of reverse or RBA banding, by the fluorescence of the largely heterochromatic long (or q) arm (27). In sexing assays the detection of two X chromosomes or one Y chromosome is considered sufficient indication of sex (28).

The accuracy of sexing using this method is nearly always 100%. The difficulty lies in obtaining biopsies which show high quality metaphase spreads of chromosomes (26). Further, the procedure requires trained cytogeneticists and is time-consuming. For example, approximately 5 h are needed for two experienced cytogeneticists to process 12 to 15 embryos (4). To circumvent these difficulties, three types of procedures using bovine embryos have been attempted, with the ultimate objective of generating a method suitable for commercial use. In the first, chromosome analyses were carried out on trophoblast cells removed from bovine embryos between Days 12 to 15. In a summary of the results of five laboratories using this technique, Betteridge et al. (29) reported that 68% of 353 embryos could be sexed, and that 33% of 110 sexed embryos resulted in pregnancy. The advantage of this procedure is that the percentage of sexable biopsies is quite high, due to the relatively large number of cells that can be removed from the embryos. However, since hatched blastocysts cannot be frozen prior to transfer, the time required for analysis makes it difficult both to obtain a result from the sexing procedure and transfer the embryos on the same day (26).

The second type of experiment involved removing small biopsies of Day 6 to 7 bovine embryos, a period which corresponds to the time when embryos can be collected, frozen and transferred nonsurgically (26). After sampling 8 to 10 cells from bovine morulae, Moustafa et al. (30) found that 59% of 44 embryos could be identified as to sex. Calving rates obtained with sexed morulae have not been well documented; however, in the study of Moustafa et al., two embryos that were transferred surgically failed to give rise to pregnancies, while all five embryos transferred nonsurgically resulted in healthy calves. The advantage of this procedure is that the restraints imposed by the time needed for sexing are not critical because embryos can be frozen prior to transfer. However, limitations are imposed by the small number of cells undergoing mitosis that are available to generate metaphase spreads sufficient for diagnosis (26). Because cells from morula-stage embryos can be grown on a collagen layer for 5 to 6 d (31), this technique could be improved by freezing the embryo and growing the cells of the biopsy until they are sufficiently numerous for cytogenetic analysis.

The third type of experiment involved splitting Day 6 to 7 embryos such that one half was frozen while the other was used for cytogenetic analysis. Using this technique Picard et al. (28) found that approximately 60% of 36 embryos could be sexed. Of the sexed embryos, 8 twin halves which were immediately transferred gave a calving rate of 60%, while 28 halves that were frozen, thawed and then transferred gave a rate of 23%. Interestingly, morphologically "good" embryos have a greater success rate for sexing then "poor" embryos. Poor embryos and all frozen demi-embryos possess a lower number of cells, and thus are more difficult to sex and/or regenerate less effectively following embryo transfer. In the case of the frozen demi-embryos, this is partly due to poor cryopreservation methods, which result in cell damage. One must also consider that a potential twin calf is lost to the sexing analysis.

In conclusion, while the cytogenetic sexing analysis itself is almost always without error, the adaptation of the procedure to the embryo transfer industry generates difficulties. The costly requirement for experienced cytogeneticists is a further deterrent. However, even if the cytogenetic technique does not find commercial application, it can be used to confirm the results of alternative developing technologies for embryo sexing.

Embryo sexing using Y-specific DNA probes. The Y-specific probe technique involves the biopsy of a small number of cells from the embryo, and hybridization of total cellular DNA from these cells to a labelled sequence of DNA that is specific to the Y chromosome. Positive hybridization results indicate the presence of the Y chromosome and thus the male sex of the embryo. The very small amount of material required for DNA preparation is the most attractive feature of the procedure; although technically an invasive method, the Y-specific probe method does not present the same problems in this regard as does cytogenetic testing.

The technology of sexing by way of Y-specific probes evolved from studies of the human testis-determining factor (TDF), which is involved in testicular development; the gene for TDF is proposed to be located on the Y chromosome. The argument for the existence of TDF stems from the fact that male sexual development requires the Y chromosome, as can be seen from individuals with abnormal numbers of X chromosomes and one Y chromosome who are nevertheless phenotypically male. The search for the location of the TDF gene on the Y chromosome led to the isolation of Y-specific DNA, which was used to probe DNA from cells of human XX males and XY females, to see which portions of the Y chromosome in these individuals were present or absent, respectively (32-34). Perhaps the ideal Y-specific probe for sexing would contain TDF gene sequences (8), although essentially any Y-specific sequence is sufficient, providing adequate controls are present. In humans, the TDF gene appears to be located on the short arm of the Y chromosome, proximal to the pseudo-autosomal region, and codes for a DNA/RNA binding protein (34). The research into TDF generated considerable information about the human Y chromosome and its relationship to the Y chromosome of other mammalian species, and resulted in the isolation of Y-specific DNA.

Unlike the X chromosome, the Y chromosome bears few known genes. In humans these genes include TDF (34), H-Y antigen (18), a gene for the antigen 12E7 (an antigen defined by a monoclonal antibody that was raised against human leukemia T cells; 35), genes for two polypeptides of unknown function (36) and possibly genes involved in the regulation of spermatogenesis (37) and tooth size (38). These genes are all found in euchromatic regions on the short arm of the Y (Yp) and the proximal end of the long arm (Yq), excluding the centromere (39). The distal region of Yp, including the telomere, has been termed the pseudo-autosomal region because pairing of this region with the X chromosome occurs during male meiosis. Sequence homology is limited, however, and there is no definitive evidence for regular crossing over in this region (35). With the exception of the proximal portion of the long arm, Yq is largely heterochromatic. Digestion of this DNA with the enzyme Hae III produces 2.1 and 3.4 kilobasepair (kbp) repeated sequences. Both types of Hae III fragments are composed of non-Y-specific reiterated sequences interspersed with Y-specific repeated elements (37). There also appear to be regions in Yp which show up to 99% homology with Xq (the DXYS loci). These are believed to be the result of an X:Y translocation in recent primate evolution (40). In fact, many Y sequences have homologous sequences elsewhere in the genome, often on the X chromosome (35). Therefore, probes corresponding to the Y chromosome must be carefully screened prior to use in sex diagnosis to ensure that the sequences are Y-specific. This step is even more critical in livestock embryo sexing, in which much less is known of potential sequence homologies of the Y chromosome to other chromosomes than is known in humans.

The generation and characterization of Y-specific DNA probes requires the following steps: isolation of Y chromosomal sequences, isolation of Y-specific sequences, determination of sequence copy number and localization of the probe sequence on the Y chromosome.

Y chromosomes are isolated routinely following either flow cytometry or interspecies somatic cell hybridization. Flow cytometry involves isolating metaphase chromosomes, staining them with a fluorescent dye, such as ethidium bromide or Hoechst 33258, and allowing them to flow individually past a laser beam (37). Because emission of the fluorescence is proportional to DNA mass, a fluorescence distribution histogram can be generated with chromosomes of a known size corresponding to specific peaks in the histogram. Y chromosomes are thus isolated, digested by restriction endonucleases and cloned into vectors to create Y chromosome-enriched libraries. In human medical research sex chromosome-enriched libraries are often prepared from cell lines of individuals with supernumerary X or Y chromosomes (for example, XYYY). Such karyotypes are rare in livestock.

Somatic cell hybrids result from the fusion of two cells of different species. The excess number of chromosomes causes instability so that chromosomes are lost at random over time during subsequent cell division. For the purpose of isolating Y chromosomes, a hybrid is used which has retained the Y chromosome of interest against a background of chromosomes from a different species. The DNA from the hybrid is isolated, partially digested, and cloned into a cosmid vector (which allows cloning of large fragments of DNA). Probing the library with a repetitive sequence specific to the DNA of the species of interest then allows isolation of the clones containing the Y chromosome fragments.

The DNA fragments in the cosmid clones must be reduced in size before they can be used to generate Y-specific probes. The Y chromosomal library is thus hybridized to male- and female-specific DNA probes to allow selection of the most Y-specific clones. Inserts from clones which hybridize strongly to only the male-specific probes are then digested with restriction endonucleases and subcloned to generate a second library. To isolate Y-specific sequences from this second library, the DNA inserts are radioactively-labelled and then hybridized to blots of DNA from male or female cells. Supernumerary bands appearing on blots generated from male DNA relative to female DNA demonstrate that the probe generated from the Y-enriched library is at least partially Y-specific. The probe is only totally Y-specific if hybridization to female DNA does not occur.

Although the ideal probe would be totally Y-specific, in practice most commercial probes used in sex diagnosis are only partially Y-specific because they hybridize to the repeated sequences on Yq, which are interspersed with non-Y-specific DNA.

Copy number for the genomic sequence corresponding to a specific probe is estimated by densitometric analysis (39). Briefly, the intensity of the hybridization signal of the probe to a blot of known genome copy numbers of normal male DNA is compared with the intensity of the hybridization signal of the probe to known copy numbers of itself.

Regional mapping of the probe can be carried out by in situ hybridization of the probe to stained spreads of chromosomes (36) which are then examined under the microscope. Because the Y chromosome is very small, the exact position of autoradiographic grains can be difficult to determine when radionuclide labels are used; other labeling techniques could be more definitive. When numerous probes are involved, pulse-field gel electrophoresis can be used to order the probes with respect to each other on the Y chromosome. This electrophoretic technique, which involves pulsed inverted or orthogonally arranged alternating electric fields, allows the separation of much larger fragments of DNA than does conventional continuous field gel electrophoresis (41). To order the probes, Y chromosomal DNA is digested with a restriction endonuclease which cuts infrequently, such as Not I, to give large fragments of DNA. Following pulse field gel electrophoresis, blots of the Y chromosomal DNA are generated and probed successively with the various probes of interest. The hybridization of probes for single copy sequences to the same fragment of DNA determines that these probes are adjacent to each other on the Y chromosome. Similarly, the hybridization of a single probe to two fragments of DNA on the blot indicates that these DNA fragments are adjacent on the Y-chromosome. When the data from a succession of individual probe hybridizations are correlated, the probes can be ordered with respect to each other, thus roughly determining their location on the Y chromosome.

Numerous human Y-specific probes have been generated in studies seeking the location of TDF. The majority of probes correspond to the euchromatic region of the Y chromosome, although probes have also been generated that are homologous to the heterochromatic portions of the Y chromosome. Probes corresponding to the 3.4 and 2.1 kbp repeated sequences on Yq fall in the latter category and are representative of many of the commercial probes used in human prenatal sex determination. Interestingly, in livestock sexing assays, such as those used in the study by Bondioli et al. (42) discussed below, probes have been generated de novo from bovine DNA rather than selected from the array of human Y-specific probes available and tested for interspecies sequence homology. As human genome sequencing and mapping continues, the number of human Y-specific probes will likely increase, as will the use of such techniques as pulse field gel electrophoresis and chromosome walking (a technique which allows the successive exploration of DNA regions adjacent to known sequences). Once reliable Y-specific probes for livestock embryo sexing are identified, they will probably be patented and used repeatedly, such that the industry may well develop on the basis of a very limited number of probes.

The first clinical application of Y-specific DNA probes was to human prenatal sex determination (43). The objective of these studies was to develop a method whereby fetuses at risk from X-linked conditions could be screened, and, in the case of a male child, perhaps aborted. Because procedures used for livestock sex diagnosis by DNA hybridization have been adapted largely from those developed for human sexing, these methods for humans will be discussed to aid in the assessment of their ultimate value to commercial livestock embryo sexing.

The first attempt at prenatal sex diagnosis by hybridization involved the use of a probe specific for the 3.4 kbp repeat sequence on Yq and Southern blots of Hae III-digested DNA prepared from chorionic villi (43). The probe bound to a number of sequences in both the male and female genome but was found to be male-specific for a 3.4 kbp fragment (only for the Y chromosome did Hae III cut the DNA to give some 3.4 kbp-long pieces containing the

repeated sequence, thus allowing sex diagnosis). Following termination of the pregnancies, sexing results were confirmed by cytogenetic analysis of cells from the fetus. Sex diagnosis was found to be correct in each of 13 cases.

In the development of the sexing technique for commercial use, Southern blotting has largely been replaced by dot blotting. In this procedure, DNA prepared from a biopsy of cells is spotted directly onto nitrocellulose, thereby eliminating the time required to separate DNA fragments of different size by electrophoresis and to transfer the DNA subsequently to a membrane. The dot blot is then probed with a Y-specific DNA sequence as before. Because dot blots do not distinguish the size of the hybridizing genomic sequence, ambiguities could arise. For example, if the probe is only partially Y-specific, binding to DNA from other chromosomes will occur. Relative to binding shown by female DNA, male DNA would show increased hybridization to the Y-specific probe due to binding to sequences from the Y chromosome in addition to binding to other chromosomal sequences. As a control, a second hybridization with an autosomal DNA probe is carried out to ensure that each dot consists of a fixed amount of DNA. The autosomal probe is expected to produce an equivalent hybridization signal independent of the origin of the DNA because the sequences to which this probe binds are not sex-specific. Using a Y-specific probe consisting of a sequence from the 2.1 kbp repeat and a probe from the Alu family as a control, Gosden and colleagues (7) correctly identified the sex of each of 77 fetuses following biopsy of chorionic villi.

Further developments in the technique of sexing using Y-specific probes have been the simplification of the genomic DNA preparation procedure and the search for a suitable single sequence probe. With regard to the preparation of DNA, Lau et al. (8) were able to obtain sufficient DNA for a dot blot by merely centrifuging cells from 0.2 ml of amniotic fluid and heating the cell pellet with an alkaline solution. This eliminates the need for repeated phenol/chloroform extractions in the conventional method of DNA preparation. Using a Y-specific probe derived from the 3.4 kbp repeats of the Y chromosome and an Alu probe as a control, they correctly determined antenatal sex using 12 amniotic fluid samples. Sex was confirmed by chromosome analysis of cultured amniotic cells.

Less success has been achieved with regard to isolating a single sequence probe. In contrast to the repeat sequence probes, which are interspersed with non-Y-specific DNA, many of the single sequence (euchromatic) portions of the Y chromosome are entirely Y-specific. A probe corresponding to these regions would thus bind only to Y chromosomal DNA, a major advantage in sex selection assays. Further, in humans, the portion of the long arm of the Y chromosome which contains repeated sequences is highly heteromorphic and may be involved in an X:Y translocation without resulting sex reversal (9), thereby giving false positive or negative results in a sexing assay. The limitation of the unique sequence probe is the reduction in the strength of the hybridization signal. Vergnaud and colleagues (9) used a Y-specific probe corresponding to the euchromatic sequences on Yp in conjunction with a repeat sequence probe from Yq in the analysis of DNA from biopsies of chorionic villi. Although sex determination was correct in each of the 12 cases, the hybridization signals resulting from the unique sequence Yp probe were difficult to interpret. The probe appears to hybridize well to the DNA from some males but not from others, and so is unlikely to be used for prenatal sex determination. A search for a suitable unique sequence probe is currently being conducted (9).

Data concerning sex diagnosis using DNA hybridization are still limited in livestock species. Bondioli et al. (42) reported the use of three repeated Y-specific DNA probes to sex Day-6 to Day-7 bovine embryos. The three Y-specific sequences are repeated approximately 2600, 600 and 20 times each per bovine genome. A fourth probe was generated from a DNA sequence that is repeated approximately 250,000 to 300,000 times in the bovine genome and which is not sex-specific. In each of two experiments, DNA was extracted and purified from a small sample (approximately 10 cells) from each embryo and the embryos were frozen during the sexing process. In the first experiment, sex was determined for 76% of 101

embryos; DNA from the remaining embryos was insufficient for analysis. Seventy-three of 77 sexed embryos were thawed and transferred, with a resulting pregnancy rate of 44%. Surgical removal of the fetus at 50 to 60 d of gestation determined that sex had been accurately diagnosed in each case. In the second experiment, sex was determined for 85% of 747 biopsied embryos. However, records of pregnancies and calvings were available for only 111 of the sexed embryos. Forty-five of the 111 sexed embryos (41%) resulted in pregnancies following thawing and transfer of the embryos. Live calves were born for 37 of the 45 pregnancies, and sex had been determined accurately for each calf.

In an analogous study, Leonard et al. (44) used in situ hybridization of a biotinylated bovine Y chromosome-specific DNA probe, referred to by Popescu et al. (45) as being 2000 to 2500 repeats of a Y-specific sequence. Fifty-seven percent (85/150) of embryos provided biopsies that yielded results from the sexing procedure. Subsequent cytogenetic analysis determined that sex had been assigned correctly for each embryo. When a limited number of Day-14 embryos were examined, the sex of seven out of eight embryos was shown to have been determined accurately.

The biotin-labelled probe used by Leonard et al. (44) is in contrast to the radioactive probes used in the studies discussed earlier. Biotin-labelled probes operate by the specific binding of the biotin to a second molecule, such as streptavidin, which is bound in turn to a reporter enzyme; hybridization is visualized by the coloured product of the enzyme reaction. Various nonradioactive methods of labeling are being developed vigorously, because the requirement for sophisticated equipment, highly trained personnel, and chemicals of limited half-life and potential hazard can be obviated.

Because mistakes cannot be allowed in a routine sex determination procedure in humans, the objective must be to develop a unique sequence Y-specific probe (preferably on Yp) so that fetuses with chromosomal aberrations can be sexed without doubt. Y-specific probes for livestock sexing are likely to be derived from repeated sequences, initially and because little is known of the exact structure of the Y chromosome and because repeated sequences are easier to isolate. Further, repeat sequence probes produce stronger hybridization signals, and so require the isolation of less DNA and therefore fewer cells. The disadvantage of repeat sequence probes, however, is that they are not entirely Y-specific and so will always bind to additional DNA on other chromosomes. The recent advent of the DNA polymerase chain reaction, an in vitro method which involves repeated cycles of primer-directed enzymatic amplification of DNA (46), may provide a means by which consistent hybridization signals can be generated from even single sequence probes; numerous copies of the probed DNA can be generated, thereby increasing the intensity of the hybridization signal. Further improvements to the sexing technique would include the development of probes with greater Y chromosome specificity, so that less ambiguity arises; better cryopreservation techniques, so that the pregnancy rate of biopsied embryos reaches the value for routine embryo transfer; and the development of effective nonradioactively-labeled probes.

Increased knowledge of the Y chromosome will also lead to more information about the proteins coded by the Y chromosome, and thus possibly, to the development of new assays for sexing such as the immunoreaction of embryos with a sex-specific antibody.

EVALUATION OF EMBRYO SEXING METHODS FOR COMMERCIAL USE

A routine procedure for embryo sexing must include both an accurate sexing procedure and successful embryo transfer. Ideally, the success rate for both requirements would be 100%, but results from the studies discussed above illustrate the potential cumulative reduction in the proportion of embryos that are sexed accurately and subsequently transferred successfully. From a commercial stand-point, the sexing method must also be easy to use and inexpensive to operate. With these factors in mind, some general comments can be made about the feasibility of each sexing technique for

commercial use.

Providing that acceptable pregnancy rates can be achieved using immunologically (H-Y antigen) sexed embryos (a feature that remains to be proven), this method shows perhaps the greatest potential for immediate use because it is rapid (2 h) and relatively simple (4). The development of an effective enzyme assay for visualization of bound antibodies will make the method even more appealing, since a kit could be prepared similar to those currently available for cow-side milk progesterone assays.

Cytogenetic analysis produces highly accurate sexing results; however, only 60 to 70% of embryos can be sexed, and the technique takes a long time - a team of two cannot be expected to analyze more than 12 to 15 embryos per day - and requires trained cytogeneticists (4). Thus, even with the development of better cryopreservation techniques for biopsied or split embryos, such that acceptable pregnancy rates can be achieved, the technique is likely to have little commercial appeal. However, cytogenetic analysis is useful for confirming results from other sexing procedures without the need for fertility trials, so that the technique will likely be useful to researchers.

The major problem in using X-linked enzymes is the potential toxicity of the assay, which would reduce embryo viability, and the dearth of information on X-inactivation. These problems may be eliminated by further research; for example, carbon dioxide generation can be monitored as a function of enzyme activity (14, 47), thus avoiding the need to stain or biopsy embryos. The technique is appealing commercially because the control assay, which measures the activity of a non-X-linked enzyme, can also be used as an index of embryo viability (14).

With respect to DNA hybridization, more work needs to be done in developing highly specific Y chromosome probes, to eliminate ambiguity, and more basic research is required on the Y chromosome in agriculturally important species. Further, more fertility trials are needed to determine the effect of the sexing procedure on embryo viability. However, the technique has shown a high degree of accuracy in prenatal sex determination in humans and is appealing because of the very small amount of tissue that is required relative to that for most invasive methods. Innovations such as nonradioactively labeled probes will likely make the technique more accessible for general use.

In conclusion, while no one method fulfills all the criteria for a successful commercial method of embryo sexing, the results from current studies suggest that with additional research a routine embryo sexing procedure may be available to the livestock industry in the near future.

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HORMONAL STUDIES OF PYOMETRA IN THE COW

G.H. Vighio[1], R.M. Liptrap[1], W.G. Etherington[2]
[1]Department of Biomedical Sciences and [2]Department of Population Medicine, University of Guelph

SUMMARY

Ovarian steroids, luteal oxytocin and uterine prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) interact to regulate luteolysis in the cow. Since persistent corpora lutea are characteristic of cows with pyometra, the positive feedback relationship between oxytocin and $PGF_{2\alpha}$ was compared between normal and pyometric animals. In experiment 1, ten cows were injected with oxytocin (0.33 iu/kg bodyweight, im) on days 5, 10 and 15 of the cycle and the $PGF_{2\alpha}$ response, as reflected in levels of 13,14-dihydro-15-keto-$PGF_{2\alpha}$, was compared to 10 similarly treated cows with pyometra. Oestrous cycle length and oestradiol and progesterone concentrations did not appear to be affected by the single injections of oxytocin on the 3 days in the cyclic cows. Progesterone and oestradiol values in cows with pyometra were similar to those of normal cows but the peak value and the area under the curve of the pulse of PGFM resulting from oxytocin injection were significantly greater in the cases of pyometra. In experiment 2, conversely, 5 cyclic cows at day 15 and 7 post-parturient cows with pyometra were injected with synthetic prostaglandin (cloprostenol, 500 μg,im) to compare oxytocin values in frequently collected blood samples. Normal cows injected with prostaglandin on day 15 secreted 70.3 $\pm$ 27.7 pmol/L/h of oxytocin compared to 4.8 $\pm$ 5.4 pmol/L/h ($p < 0.01$) seen in cows with pyometra. The persistent corpora lutea of cows with pyometra is not due to an insufficiency in synthesis of uterine $PGF_{2\alpha}$ but may be associated with inadequate secretion of ovarian oxytocin.

INTRODUCTION

Bovine pyometra occurs most commonly in the early postpartum period and is characterized by the accumulation of mucopurulent exudate in the uterus, persistence of the corpus luteum and anoestrus (Olsen et al., 1986). Retention of the corpus luteum is believed to be due to a failure in the pulsatile release of uterine prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) which is controlled by an interaction with luteal oxytocin and ovarian steroids. Despite elevated concentrations of both prostaglandins F and E in the uterine material (Manns et al., 1985) cows with pyometra seem to secrete greater amounts of progesterone than do normal cows at a comparable period postpartum (Mortimer et al., 1983).

The plasma values of oxytocin and prostaglandin are highly correlated during spontaneous luteolysis (Vighio and Liptrap, 1986) and it has been suggested that a positive feedback loop between ovarian oxytocin and uterine prostaglandin leads to a more rapid decline in corpus luteum function (Flint and Sheldrick, 1983). Oxytocin administration increases circulating levels of $PGF_{2\alpha}$ (Newcomb et al., 1977) and its metabolite 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM) in the cow (Oyedipe et al., 1984). Conversely, oxytocin reaches peak values 30 minutes after injection of $PGF_{2\alpha}$ analogue (Schams and Karg, 1982). The purposes of this study were twofold: (i) to compare the PGFM response between normal cows and cows with pyometra to a standard dose of oxytocin and (ii) to compare the amounts of oxytocin secreted in response to $PGF_{2\alpha}$ injection between the two groups.

MATERIALS AND METHODS

In experiment 1 ten normal, cyclic Holstein cows were used to establish the plasma PGFM response to a standard dose of oxytocin (0.33 iu/kg bodyweight, im) administered on days 5, 10 and 15. Ten lactating cows with clinical signs of pyometra were similarly examined. In experiment 2, five normal cows received 500 μg of the synthetic prostaglandin, cloprostenol (Estrumate, Upjohn Co., im) on day 15 of the cycle. An additional 7 cows with pyometra received a similar prostaglandin injection to compare the oxytocin response between the two groups. Cows with pyometra were 35 to 65 days postpartum. Uterine horn diameter ranged from 3 to 14 cm and A. pyogenes, and occasionally E. coli and Streptococci organisms were most commonly

identified in uterine material. The same blood sampling regimen through an indwelling jugular catheter was used in all animals. Pre-injection samples were taken at 15 minute intervals from 08.00h to 09.00h and after oxytocin or prostaglandin injection were taken at 15 minute intervals from 09.00h to 12.00h and at 30 minute intervals from 12.00h to 15.00h. Twice daily samples were also collected throughout the cycle in the normal cyclic cows examined on days 5, 10 and 15.

PGFM concentrations were measured by radioimmunoassay (Fairclough and Payne, 1975) as previously described (Liptrap, 1980). Radioimmunoassay procedures were used for the estimation of progesterone (Abraham et al., 1971) and oestradiol (Edqvist and Johansson, 1972) as outlined before (Liptrap, 1980). Plasma values for oxytocin were determined by radioimmunoassay (Gorewit, 1979) as conducted previously (Vighio and Liptrap, 1986).

Variables describing PGFM and oxytocin pulses after hormone injection were assessed by computer analysis (Santen and Bardin, 1973) as modified by Clifton and Steiner (1983). Two way analysis of variance was used to examine for statistical differences between animals and between days for each of the three hormones measured (Steel and Torrie, 1980). Student's t-test was used to compare mean hormone values of the pyometritic cows with those of the normal cyclic cows on each of the test days studied in the two experiments.

RESULTS

Oestrous cycle length was not altered in the cows given a single oxytocin injection on days 5, 10 and 15 in the first experiment. Oestradiol and progesterone concentrations in plasma collected twice daily are shown in Figure 1. A summary of the hormonal variables calculated for the cows receiving oxytocin injection is given in Table 1. Apart from basal concentrations, cows with pyometra secreted significantly more prostaglandin as reflected in metabolite values than did normal cyclic cows in response to oxytocin injection. Plasma progesterone values increased significantly between days 5 and 10 but the values present in pyometritic animals did not differ statistically from those of cyclic cows on days 10 and 15. No differences between days or groups were seen for oestradiol concentrations. The calculated pulse area for oxytocin in plasma after injection of oxytocin was 190 $\pm$ 71 pmol/L above base line values (mean $\pm$ S.D.) with peak values of 130.2 $\pm$ 121.4 pmol/L occurring 45 minutes after administration.

Figure 2 shows the effect of prostaglandin injection upon steroid and oxytocin concentrations for the second experiment in normal cows at day 15 and in cows with pyometra. Although no significant differences were present between the two groups for oestradiol and progesterone values, a significant difference ($p < 0.01$) did exist for the stimulated pulsatile release of oxytocin. Cyclic cows secreted a mean pulse value of 70.3 $\pm$ 27.7 pmol/L/h with a mean peak value of 141.7 $\pm$ 51.2 pmol/L/h compared to 4.8 $\pm$ 5.4 and 25.6 $\pm$ 2.9 respectively for cows with pyometra.

DISCUSSION

Although a brief partial drop in progesterone concentrations was seen in individual cows following injection of oxytocin, these recovered quickly and luteal function, as reflected in the twice daily progesterone values, appeared to be unaltered. Steroid hormone values were similar to those previously reported for the cyclic cow (Wettemann et al., 1972, Glencross et al., 1973). Seemingly, multiple doses of oxytocin are required in order to affect luteal activity and shorten the oestrous cycle (Donaldson and Taaken., 1968, Oyedipe et al., 1984).

The mean values for variables characterizing the PGFM pulse on the 3 control days of the cycle were not statistically different. Using a smaller dose of oxytocin, Lafrance and Goff (1985) did not induce a significant increase of PGFM until the time of luteolysis on days 17 to 19.

Baseline values for PGFM were not different between normal animals and those with pyometra, although higher basal concentrations in the latter have been reported (Watson, 1984, Manns et al., 1985). The amount of PGFM present after oxytocin injection

in pyometritic cows was greatly exaggerated compared to the 3 control days examined and the mean peak value was about 4 times that seen during spontaneous luteolysis (Vighio and Liptrap, 1986). Persistence of the corpus luteum characteristic of cows with pyometra is not due to a deficiency in the uterine synthesis of prostaglandin. In fact, amounts of PGF and PGE far above normal have been reported in uterine fluid obtained from clinical cases of pyometra (Manns et al., 1985).

The other important aspect of luteolysis in the cow involves ovarian oxytocin secretion (Flint and Sheldrick, 1983). Ovarian oxytocin content is highest about days 5 to 10 (Wathes et al., 1984) and the greatest secretory response to PGF stimulation in vitro, was seen from tissue collected in the middle of the cycle (Abdelgadir et al., 1987). Nevertheless, peak values between 100 and 200 pmol/L were observed in cows injected on day 15 of the cycle with prostaglandin. Similar results have been reported previously (Shallenberger et al., 1984). In contrast, almost no oxytocin was secreted by cows with pyometra following a similar stimulation. These animals, with prolonged luteal function, are reminiscent of pregnant cows whose corpora lutea have undetectable amounts of oxytocin (Wathes et al., 1984).

Collectively, the present results suggest that the persistent corpus luteum of postparturient cows with pyometra is not due to inadequate synthesis of uterine prostaglandin but rather appears to be associated with a deficiency in ovarian oxytocin. The ability of the ovary to produce oxytocin in the earlier, developmental stages of pyometra is not known and further studies on ovarian oxytocin regulation seem warranted.

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Table 1: Summary of hormone variables (mean ± S.D.) calculated for 10 normal cows on days 5, 10 and 15 of the estrous cycle and for 10 post parturient cows with pyometra after injection of oxytocin (0.33 iu/kg bodyweight im).

Hormone Variable	Cyclic Control Cows			Pyometra
	day 5	day 10	day 15	post partum
PGFM				
pulse area (pmol/ml/h)	137.5±109.2[a]	143.3±109.2[a]	315.8±260.4[a]	3515.9±4888.8[b]
pulse duration (h)	1.8±0.5[a]	2.1±0.8[a]	2.3±0.6[a]	2.6±0.9[a]
pulse peak (pmol/ml)	423.9±123.2[a]	571.2±159.6[a]	612.0±534.8[a]	2343.6±1401.9[b]
basal value (pmol/ml)	302.4±103.6	377.7±128.8	343.8±190.4	550.5±850.6
mean value (pmol/ml)	339.6±109.2[a]	411.0±148.4[a]	389.5±226.8[a]	850.6±349.2[b]
$\bar{x}$ Protestosterone (nmol/L)	9.2±5.7[a]	19.7±10.2[b]	27.3±10.5[b]	26.2±9.5[b]
$\bar{x}$ Estradiol (pmol/L)	36.3±23.8	37.8±20.9	31.6±21.3	25.7±2.9

a,b different superscripts indicate statistically different means

Figure 1. Plasma concentrations (vertical lines indicate S.D.) of oestradiol and progesterone in 10 cows that received oxytocin injections (0.33iu/kg bodyweight, im) on days 5, 10 and 15 (arrows) of the cycle.

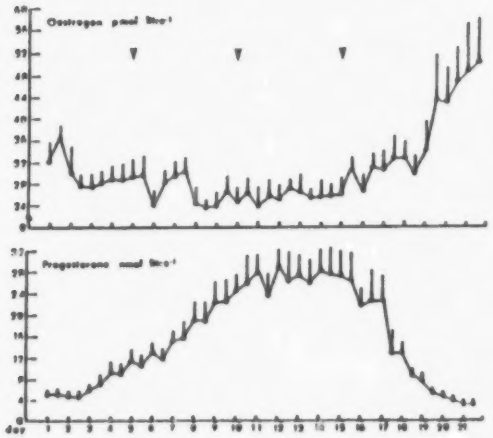
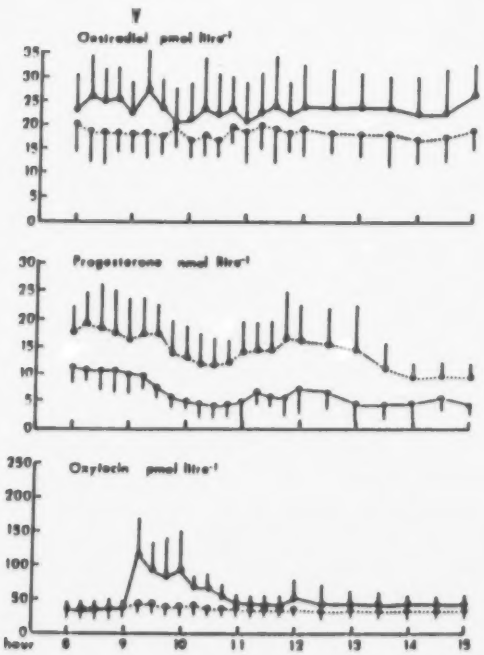


Figure 2. Plasma concentrations (vertical lines indicate S.D.) of oestradiol, progesterone and oxytocin in 5 normal cows (solid lines) on day 15 and 7 cows with pyometra (dotted lines) that received an injection of synthetic prostaglandin (cloprostenol, 500 g, im).



EFFECTS OF PROGESTERONE AND HUMAN CHORIONIC GONADOTROPHIN ADMINISTRATION FIVE DAYS POST INSEMINATION ON PLASMA AND MILK CONCENTRATIONS OF PROGESTERONE AND PREGNANCY RATES OF NORMAL AND REPEAT BREEDER DAIRY COWS

J.S. Walton[1], G.W. Halbert[2], N.A. Robinson[1], and K.E. Leslie[2]
[1]Department of Animal and Poultry Science and [2]Department of Population Medicine, University of Guelph

SUMMARY

Treatment with a progesterone-releasing intravaginal device (PRID) between days 5 and 12 after estrus elevated ($P<0.05$) plasma progesterone concentrations between days 6 and 8 in comparison with controls. Treatment with injectable progesterone (200 mg) on days 5, 7, 9 and 11 post estrus did not increase plasma progesterone concentrations over controls. The administration of 1500 iu human chorionic gonadotrophin (hCG) on day 5 after estrus resulted in a sustained increase ($P<.01$) in plasma progesterone concentrations from day 8 until day 20 when measurements ceased. Pregnancy rates, as a result of AI at the preteatment estrus, in these treatments (n=12-14), were unaffected by any of the treatments and ranged from 57.1 - 75.0%. In a field trial, of 36 repeat breeder cows treated with 1500 iu hCG 5.5 days after insemination, 47.2% were pregnant at 60 days, whereas 39.5% of saline-treated controls were diagnosed pregnant. Treatment with hCG significantly ($P<0.05$) increased milk progesterone concentrations over controls on days 14 and 20 after insemination. The use of a cow-side progesterone assay (Target) at day 20 indicated a predictive value of 87% for non-pregnancy, but only 58% for pregnancy because of a large number of elevated progesterone concentrations in cows which were diagnosed open at a 60 day rectal palpation.

INTRODUCTION

It is often considered that early embryonic mortality is responsible for a substantial proportion of pregnancy failures, especially in cows which have already received a number of inseminations. Therapeutic treatment often involves the administration of progesterone in an attempt to assist in the support of the early stages of pregnancy. For example, we have previously demonstrated that progesterone-releasing intra-vaginal devices (PRID) were effective in increasing circulating progesterone concentrations and pregnancy rates when placed in the vagina of dairy cows between days five and twelve or days twelve through seventeen post-insemination. At the same time, it was also demonstrated that supplementation with progesterone is often negated by a tendency of the animals under treatment to reduce progesterone synthesis, especially later in the cycle. Given that PRID are unavailable to dairy farmers in North America and exogenous progesterone supplementation is unlikely to be a fruitful approach to treatment, we decided to investigate alternate treatments to increase circulating progesterone concentrations and improve the opportunity for cows to remain pregnant.

Initially, a preliminary experiment was conducted to compare alternate methods for elevating plasma progesterone concentrations using injected progesterone, human chorionic gonadotrophin (hCG), and PRID in comparison with untreated controls. On the basis of these results, a field trial on the effectiveness of hCG treatment in raising milk progesterone concentrations and pregnancy rates was conducted.

MATERIALS AND METHODS

Lactating Holstein cows at the Elora Dairy Research Centre, University of Guelph, were randomly allocated to treatment after the first artificial insemination of the breeding season (60 to 80 days postpartum). Fourteen control animals received no treatment, thirteen animals had a PRID (CEVA Laboratories, Kansas, estradiol capsule removed) inserted on day five post-AI followed by removal on day twelve. Twelve cows were treated with an injectable progesterone (Centra Progestin, Central Sales Ltd., Brampton, Ontario) at a dose of 200 mg/animal on days five, seven, nine and eleven post-insemination. Fourteen cows were

treated with 1,500 iu hCG (Progon, PVU Inc., Victoriaville, P.Q.) on day five post-insemination. Cows were inseminated using semen from a large battery of proven Holstein sires which, in terms of this experiment, were randomly distributed across all treatments. For all cows, starting four days post-insemination, blood samples were taken from the coccygeal vein/artery at daily intervals until twenty-one days after insemination. To ensure cows were inseminated at the correct time, milk samples were taken and assayed for progesterone from estrus onwards and only those cows that were inseminated with base-line progesterone were included in the analysis. Cows were observed for return to service and were palpated rectally to determine pregnancy status forty-five to sixty days post-insemination. Milk progesterone determinations were continued during this period to identify the incidence of embryonic mortality and also as a check on estrous detection and pregnancy determination. Both milk and plasma progesterone concentrations were determined using previously validated radioimmunoassays and the results collated over time.

Holstein cows, from farms serviced by the Ontario Veterinary College Ruminant Health Management Clinic, that had been bred three or more times were entered into this experiment. A total of eighty-two repeat-breeder cows from twenty-four farms were ultimately included. The cows were a mean age of 5.5 years and were a mean of 150 days from parturition to the treatment service. Treatments were administered between four and six days post-insemination such that the mean interval from service to treatment was 5.5 days. The cows were randomly assigned to i.m. treatment with either 1,500 iu hCG extended to 5 ml with .9% saline or 5 ml .9% saline alone. At the time of treatment a milk sample was taken and preserved with potassium dichromate for subsequent progesterone assay by radioimmunoassay. Milk samples were also taken fourteen and twenty days post-insemination for radioimmunoassay of progesterone and pregnancy status was confirmed at rectal palpation by experienced veterinarians 45 to 60 days post breeding.

In addition to radioimmunoassay the milk samples on day twenty were also tested using the rapid qualitative enzyme-immunoassay (Target, Biometallics, Princeton, New Jersey). The results of this rapid test were evaluated as a test for non-pregnancy in addition to the subsequent rectal palpation.

Comparisons between treatments for pregnancy rates were determined using Fisher's exact test or Chi-square analysis. Comparisons among means were conducted largely using Student's t-tests, using the Bonferroni adjustment for unequal variances as necessary. All statistical procedures were conducted using PC-SAS.

RESULTS

Pregnancy rates did not differ across treatments and ranged from 57 to 75% (Table 1). Plasma progesterone concentrations in the various treatment groups are described in Figure 1. Progesterone concentrations were increased ($P<0.05$) by the administration of PRID, but only between days 5 and 8 post-estrus. Cows treated with injectable progesterone had similar ($P>0.05$) progesterone concentrations to control cows throughout. It should be noted that blood samples were taken immediately prior to treatment. The progesterone injected after blood sampling on the previous day was, therefore, not evident in the sample taken the next day. The only treatment that provoked a sustained increase in progesterone over controls was hCG. This difference ($P<0.01$) was not evident until day 8 post-estrus but was sustained thereafter. Rectal palpation of the ovaries of these animals on days 14 through 16 of the treatment cycle revealed the presence of one or two supernummary corpora lutea on the ovaries. Across all treatments, cows which did not become pregnant returned to estrus within 21 $\pm$ 3 days of their previous estrus and became pregnant to subsequent inseminations.

There were no differences in age (5.6 versus 5.3 years) or service number at the time of treatment (4.3 versus 4.0) for control versus hCG-treated cows, respectively. The pregnancy rates and milk progesterone concentrations for these animals are given in Table 2. Pregnancy rate was not different (47.2% versus 39.5%) between hCG-treated and control cows. Treatment with hCG did, however, significantly

increase milk progesterone concentrations on both days 14 and 20 post-breeding. Given the lack of treatment effect on pregnancy rates, pregnant versus non-pregnant cows were compared in terms of progesterone concentrations. There were, again, no differences between progesterone concentrations in pregnant versus non-pregnant cows on days 5 or 14 after breeding, although there was a difference ($P<0.05$) between non-pregnant and pregnant cows on day 20 with the non-pregnant cows having a lower milk progesterone concentration (Table 3).

Since it is important that the hCG treatment should not interfere with the normal return to estrus in cows which do not become pregnant, the milk progesterone concentrations for the day 20 samples were divided both into treatment and pregnancy status and these results are illustrated in Table 4. It can be observed that in non-pregnant cows, progesterone concentrations were not different between saline and hCG-treated groups. It can also be observed that the effect of hCG on progesterone concentrations persisted until day 20 in those animals which became pregnant.

The evaluation of the Target test as an indicator of non-pregnancy using the 20 d milk samples is illustrated in Table 5. Target was useful in predicting cows which were not pregnant, but was of low predictive value for pregnancy, largely because of open cows with elevated progesterone levels on day 20.

DISCUSSION

The objective of this research was to develop methods for increasing concentrations of progesterone between days 5 and 20 post-insemination. It was felt that this was an appropriate strategy because it has previously been demonstrated that pregnant heifers have higher concentrations of progesterone than non-pregnant heifers at this time, and that a quiescent, progesterone-dominated uterus is essential for the establishment of the conceptus within the uterine lumen.

As in previous studies, PRID was effective in supplementing progesterone concentrations, but only for the first 3 days after its insertion. We have previously demonstrated that the effect of PRID in supplementing progesterone is at least partially abrogated by a reduction in endogenous luteal progesterone output. Although the progesterone product that was injected was supposedly a long-acting preparation, a sustained elevation of progesterone could not be demonstrated using this product.

In terms of elevating progesterone concentrations for most of the period in question, the only successful treatment was the administration of hCG on day 5. It should be noted that the effect of hCG treatment was not apparent until day 8 post-estrus. This, together with the palpation data which indicated that these animals formed additional corpora lutea after treatment, is strongly suggestive of the following mode of action. It is proposed that the injection of hCG causes rupture and luteinization of any large follicles which are present on the ovaries at the time of treatment. Since it takes 2 to 3 days for the resulting corpora lutea to generate substantial amounts of progesterone, this would account for the delay in the increase in progesterone concentrations following treatment. In a separate study, beef heifers were treated in an identical fashion and their ovaries were recovered at slaughter on day 12 of the estrous cycle. Inspection of the ovaries revealed that each of these animals had two corpora lutea of differing age, one a 12 day corpus luteum from the original estrus, the second an induced corpus luteum approximately 4 days of age.

Despite the considerably elevated progesterone concentration in the hCG-treated animals, there was no effect of treatment with either hCG or, indeed, any of the other treatments on pregnancy rates. Any cows that did not become pregnant after hCG treatment returned to estrus at approximately the normal time, such that the treatment did not interfere with normal rebreeding.

It should be noted that the inherent fertility of this group of animals was good. In previous studies, the effects of progesterone on improving pregnancy rates were generally observed in situations where fertility was relatively poor. As a consequence, it was decided that on the basis of this first experiment, hCG treatment on day 5 was an

appropriate technique for stimulating increased progesterone concentrations during this critical phase of early pregnancy and was worthy of testing in a larger experiment using cows with compromised fertility, to determine whether it would be effective in improving pregnancy rates under field conditions.

Cows in the field trial were selected on the basis of a history of unsuccessful artificial inseminations, and were regarded as subfertile cows. Pregnancy rates in the experiment were somewhat better than expected, with 39.5% of controls and 47.2% of treated animals becoming pregnant to the treatment insemination. This may indicate that the animals selected for treatment had not become pregnant because of inadequate management practises, which were, to a certain extent, remedied by a response of producers to the implementation of an experimental protocol. However, since neither the producers nor the clinicians were aware of which cows received which treatment, this would not represent a bias in the experimental procedure.

Under these conditions, there was no effect of hCG on pregnancy rate, although hCG treatment elevated milk progesterone concentrations in milk samples taken on day 14. This effect was sustained through day 20, such that cows which became pregnant following treatment with hCG had elevated progesterone concentrations relative to cows which were not pregnant or treated with saline. These data indicate the prolonged effect of treatment, presumably because the accessory corpora lutea were sustained for a relatively long period of time. Unfortunately, these cows were not followed any further and we do not know at this time whether the accessory corpora lutea last the entire gestation.

There was also no relationship between progesterone concentrations after treatment and pregnancy status. This is, perhaps, not surprising, given the lack of treatment effect on pregnancy rate and the approximately equal distribution of pregnant and non-pregnant cows with and without hCG treatment.

The small secondary study involving the use of the Target ELISA Test for milk progesterone on day 20 indicated that this test was very similar in sensitivity, specificity and predictive value for non-pregnancy, as has been described for a large variety of radioimmunoassays and enzymeimmunoassays. It was of reasonably good predictive value as a test for non-pregnancy, although it did classify 3 animals not pregnant which turned out to be pregnant. If these cows had been subsequently treated to induce estrus, this would have meant the loss of 3 pregnancies. In terms of a test for pregnancy, the predictive value of the test was no better than simple guesswork. A large number of cows with high progesterone on day 20 using this test and radioimmunoassay turned out to be non-pregnant (22 out of 52). In previous reports, the predictive value of milk progesterone on day 20 as a test for pregnancy is normally somewhat better than this. It is normally considered that these false indications of pregnancy are related to artificial insemination at an inappropriate time, uterine pathology, or early embryonic mortality. Since these cows were checked for the absence of uterine pathology, it is most likely that false positives were related either to insemination at an inappropriate time, or they suffered embryonic mortality. If the major problem was the latter, then it would appear that the treatment with hCG did little to alleviate this problem.

In conclusion, therefore, treatment with hCG on day 5 after insemination will substantially elevate progesterone concentrations during the next two weeks. In cows which do not become pregnant, this treatment does not interfere with the normal return to estrus and is an effective way of creating supplemental progesterone concentrations in dairy cows. While the current experiments did not indicate any positive effect of this treatment on pregnancy rates, there was also no indication that it created any problems. In situations, therefore, where it may be suspected that inadequate progesterone concentrations may be involved in poor fertility of dairy cattle, then this treatment would be regarded as being superior to supplementation with exogenous progesterone if the objective is to obtain elevated progesterone concentrations for an extended period of time in response to a single treatment.

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Table 1. Effect of progesterone and human chorionic gonadotrophin (hCG) treatment on pregnancy rates in Holstein cows.

Treatment	Control	PRID[a]	Progesterone[b]	hCG[c]
No. of cows	14	14	12	14
No. pregnant	8	8	9	8
% pregnant	57.1	61.5	75.0	57.1

[a] PRID - Progesterone-releasing intravaginal device from days 5-12 post-AI.
[b] Progesterone - 200 mg Centra Progestin on days 5, 7, 9 and 11 post-AI.
[c] hCG - 1500 IU of human chorionic gonadotrophin on day 5 post-AI.

Table 2. Milk progesterone and pregnancy results in repeat breeder cows treated with hCG (1500 IU hCG on day 5 post AI) or saline control.

	Saline	hCG Treated
No. of cows	43	36
No. Pregnant (%)	17 (39.5)[a]	17 (47.2)[a]
Milk progesterone on:		
Day 5 (pre-treatment) (ng/ml)	0.9 $\pm$ 0.10[a]	1.4 $\pm$ 0.31[a]
Day 14 (ng/ml)	3.2 $\pm$ 0.30[a]	4.3 $\pm$ 0.46[b]
Day 20 (ng/ml)	2.9 $\pm$ 0.30[a]	4.1 $\pm$ 0.60[b]

[a,b] Means are different $p<0.05$

Table 3. **Milk progesterone concentrations in pregnant versus non-pregnant repeat breeder cows**

	Pregnant	Non-pregnant
Number of cows	34	45
Mean service number	3.9	4.4
Milk progesterone on :		
Day 5 (ng/ml)	1.1 ± 0.24	1.2 ± 0.19
Day 14 (ng/ml)	3.7 ± 0.42	3.7 ± 0.36
Day 20 (ng/ml)	4.1 ± 0.54[a]	2.8 ± 0.41[b]

[a,b] Means are different $p<0.05$.

Table 4. **Milk progesterone concentrations on day 20 after AI in repeat breeder cows in relation to treatment and pregnancy status**

Treatment	No. of Cows	Pregnancy Status	P_4 (ng/ml) ±SE
Saline	26	Not pregnant	3.0 ± 0.55[a]
Saline	17	Pregnant	2.7 ± 0.45[a]
1500 iu hCG	19	Not pregnant	2.5 ± 0.65[a]
1500 iu hCG	17	Pregnant	5.8 ± 0.86[b]

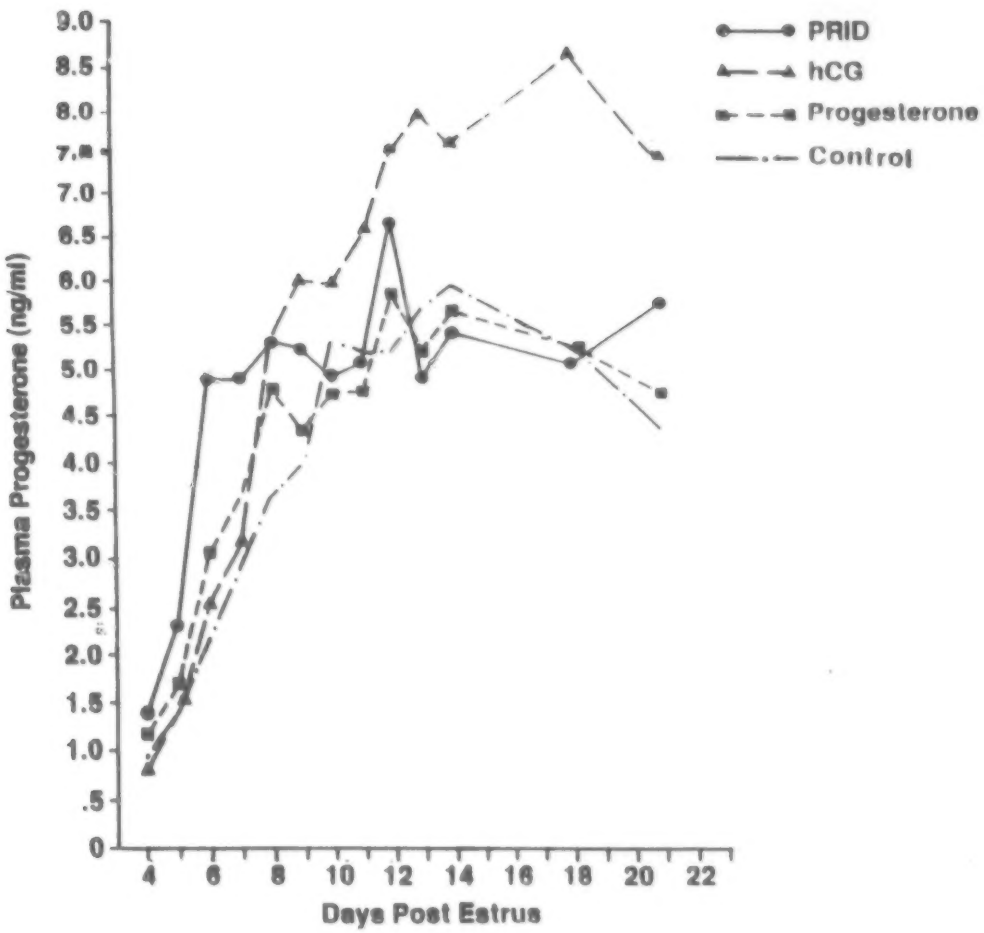
[a,b] Means are different $P<0.05$.

Table 5. **Relationships between pregnancy status at rectal palpation 45-60 days post, A1 and milk progesterone determined using a Target test on d 20.**

		Number of Cows Confirmed: Non-pregnant	Pregnant	Total
# of cows with Target test:	Low or Intermediate	20	3	23
	High	22	30	52
Total		42	33	75

Evaluation of Target test for non-pregnancy:
Sensitivity = 20/42 = 47.6%
Specificity = 30/33 = 90.9%
Prevalence of pregnancy = 42/75 = 56%
Predictive value of test for non-pregnancy = 20/23 = 87.0%
Predictive value of test for pregnancy = 30/52 = 57.7%

Figure 1. Effect of treatment with 1) PRID (progesterone-releasing intravaginal device) between days 5-12 post estrus; 2) 200 mg progesterone on days 5, 7, 9 and 11 post estrus; 3) 1500 iu human chorionic gonadotrophin (hCG) on plasma concentrations of progesterone in inseminated Holstein cows. Data from untreated controls are included for comparison; n= 12-14 across treatments. Means are different (at least $P<0.05$) from controls for PRID, days 6, 7, 8; hCG days 8-21. Pregnancy rates are illustrated in Table 1.



PROPOSAL FOR THE ESTABLISHMENT OF A CENTRE FOR THE STUDY OF ANIMAL WELFARE

Ad Hoc Committee

Membership:

R. Downey, Asst. Dean, O.V.C.
F. Hurnik, Dept. Animal & Poultry Science
D.M. Lavigne, Dept. Zoology
H. Lehman, Dept. Philosophy
D.G. Porter, Dept. Biomedical Sciences (in the Chair)

"The University will have to address a variety of issues concerning both its internal operations and aspects of society on which it can exert an influence. Its role as a leader in society will depend upon the sensitivity and wisdom it displays in approaching issues that otherwise might prove divisive. For example, it must continue to face the complex, and sometimes difficult, questions associated with the use of animals in research and teaching .... it will have to maintain a forum for debate in which all interested individuals have an opportunity to contribute fully: members of the public, alumni, board members, staff, faculty, and students. Opportunities for full participation will be especially important given the heterogeneous and multicultural composition of the institution."

Toward 2000:
Challenges and Responses
Aims of the
University of Guelph, p.10

1. INTRODUCTION

There are clear indications that society is changing its views on the relationship between humans and other animals. Segments of society have raised important questions about how humans should treat non-human animals, and the assertion of animal welfare philosophers that the criterion that should guide human conduct towards animals should be the capacity of the animal to experience a quality of life is recognized by many as a qualitative step in the evolution of human morality.

There is no doubt that many animals are treated badly by humans. In some cases suffering is caused unintentionally and is occasioned by ignorance. In others it is inflicted gratuitously in the pursuit of goals which have a human priority that is deemed to override the needs of animal welfare. In still others, suffering may be inflicted intentionally.

The problems that arise in attempting to reduce the sum total of animal suffering are complex. They may be resolved into three major inter-related domains, namely the Ethical problems which pose the question of the moral status, if any, of non-human animals and the extent to which their moral status should override human priorities, the Legal problems of establishing laws to protect animals or promote welfare, and the Practical, which involve the problems of reducing animal suffering by altering the environment of animals, for example through changes in their housing, their health, the way they are transported or killed or in the case of wild animals, the protection of their habitat.

There is a pressing need to study each of these domains because with the expansion of human population at an exponential rate and with the continued and unabated usage of animals by the human population for a variety of purposes, the total amount of animal suffering has probably increased in proportion. The same expansion of our population has also been at the expense of the natural habitat of numerous wild animals, many of which have either been brought to, or are threatened with extinction. Moreover, modern technologies have enabled many developments, such as intensive farming, gene manipulation, and chemical industries, which threaten to inflict further suffering on animals kept by, or which live in close proximity to, humans.

The University of Guelph has already contributed significantly to the perception and practice of animal welfare. It has organized international conferences on the subject of animal well-being and has been instrumental in the drafting of several farm animal Codes of

Practice now used by industries and animal welfare organizations. The University has also developed undergraduate and graduate courses (Appendix I) in the subject. Several of its Colleges: Agriculture, Arts, Biological Science and Veterinary Colleges, have faculty who have contributed in various ways to different aspects of Animal Welfare. A list of Faculty, by no means exhaustive, who have already expressed a wish to be associated with the Centre is attached (Appendix II), together with a brief statement of their respective interests. Moreover, the University's Animal Care Committee has not only fulfilled its mandate under the law, but has exhibited considerable leadership in the pursuit of animal welfare on campus (see Guidelines for the Use of Animals in Research and Teaching, Animal Care Services, University of Guelph). Inevitably, it has major roles in reviewing animal procedures on campus, and in a regulatory capacity. It is not however in a position to develop the wider activities envisaged for the Centre.

The contribution of the University to animal welfare has been extended as the University is the home of a new international scholarly journal, the Journal of Agricultural Ethics, co-edited by two faculty members, Prof. F. Hurnik, Department of Animal and Poultry Science, and Prof. H. Lehman, Department of Philosophy.

In some respects, however, the University is yet another institution with a vested interest and a long tradition in the use of non-human animals for a wide array of human activities. The University differs from other special interest groups in that it has a more important role to play in the field of animal welfare than merely reacting to pressures brought to bear upon it by various special interest groups.

Many of its members have extensive experience and expertise both in dealing with animals, and in the analysis of the important philosophical questions raised by our society. Unlike other major institutions, the University also provides, through the principle of 'academic freedom', an environment in which issues, such as those posed by the subject of animal welfare, can be freely studied and discussed without being constrained by the Institution's special interests or policies. In other words, the University has a responsibility, as acknowledged in "Toward 2000: Aims of the University of Guelph", to address the complex questions raised by the subject of animal welfare. It must do this in order to educate itself on this matter and also to ensure that its Institutional responses are based upon a broad understanding of the various viewpoints that are involved. There is, of course, a larger responsibility: to provide reasoned argument for the public debate in order that society obtains a more balanced view of the subject than can usually be gleaned from listening only to the extreme positions adopted by parties and individuals with vested interests in mind.

It is proposed, therefore, that the University of Guelph establish a Centre for the Study of Animal Welfare. Such a University Centre will be a first in Canada and one of very few in the world.

2. THE CENTRE

The proposed Centre for the Study of Animal Welfare will be organized on the basis of existing models for 'Centres' at the University of Guelph. It will be an inter-disciplinary academic unit that crosses traditional College boundaries.

2.1 OBJECTIVES

It is intended that the Centre should have as its primary tenet the improvement of the welfare of animals and that it should devote its energies and resources to the pursuit of those aspects of the subject that are likely to yield the greatest gains in animal welfare. Central to the activity of the Centre will be the philosophical analysis of the relationship of humans to non-human animals and the implications of this analysis for the varied human-animal interactions in society. Thus the Centre would have a function as a "think tank" in which the various aspects of animal welfare could be debated in an open and objective environment.

The Centre's objectives would fall into three broad categories: scholarly research, education and advisory.

It would not be appropriate at this time to specify in detail all the aspects of animal welfare upon which the Centre would focus, since much will depend upon the personality and interests of the Director and the 'core' faculty (see below), but it is possible to give some outline of the general activities that the Centre would pursue.

It would:

(1) conduct investigations to determine how maximum standards of animal welfare can be achieved.

This would be likely to involve studies to define what is meant by 'animal welfare' as well as to develop better means to assess or to measure it. It would also encompass the development of more humane systems of housing, transport and handling of animals.

(2) **conduct scholarly research**

In addition to the philosophical enquiry into the human - non-human animal relationship, this would be likely to involve, for example, studies of the compatibility of welfare promotion and agricultural productivity, the cost vs. benefit analysis of animal experimentation, the human - companion animal bond, and the humane aspects of wildlife conservation.

Research on Animal Welfare inevitably raises issues in which matters of fact and interpretation of facts become inseparably intertwined. Designing and conducting such research often requires the harmonious co-ordination of specialists who work traditionally in relative isolation from one another. Without the framework which the Centre would provide, it will remain difficult to promote such interaction and, as a consequence, important research on animal welfare will continue to be neglected.

(3) **undertake appropriate educational and advisory activities**

This might include the organization of symposia, and visiting professorships, the dissemination of information and research results both on and off campus and the participation in courses in animal welfare. Whilst it is not intended that the Centre establish undergraduate and graduate courses, these being the responsibility of Colleges, the Centre would provide intellectual support for existing courses (Appendix I) or for the creation by Colleges of new ones.

The Centre would thus serve as a catalyst for the development of courses in Animal Welfare in co-operation with other academic units.

The Centre would be expected to perform an educational function in a wide arena. The subject of animal welfare is often approached from entrenched viewpoints. In pursuing its objective of rational analysis, the Centre would be expected to assume an educational responsibility off campus. It would inform interested parties of the results of its analysis and through lectures, conferences, discussions, seminars and writings, aim to foster a more thoughtful approach to animal welfare issues.

The Centre would have an important advisory commission, providing scientific data and informed opinion based on scholarly research on a wide range of animal welfare issues to parties within the University, industries which use animals, government agencies and the animal welfare movement.

(4) **raise funds**

The Centre would have a continual commitment to fund raising, both to support its base budget and to finance its special projects.

2.2 STRUCTURE

When full strength is achieved, the University of Guelph Centre for the Study of Animal Welfare will consist of a full-time Director and at least four full-time faculty members (initially seconded from within the University). These people would constitute the 'critical mass' of the Centre. In addition, there will be a number of Associated Faculty drawn from Departments and other Centres on Campus that have special interests

in the subject. The Centre will require a number of support staff, particularly in view of the breadth and complexity of its functions (see Figure 1).

The Centre will be administered by a Director who will be guided by a Steering Committee drawn from the University Community. The Steering Committee will be expected to consult, on an annual or semi-annual basis, a wide spectrum of opinion from the various interest groups and supporters of the Centre. The purpose of this consultation will be to sharpen the focus and the direction of the Centre as well as to provide the various groups with a perspective of the Centre's operations.

As indicated above, opinions on animal welfare issues tend to be emotionally charged as the disputants frequently adopt polarized positions. The Steering Committee through its wide contacts with the different interest groups, will keep the Director briefed on their various viewpoints. It is hoped that involving groups with disparate opinions in the process of formulating policy will also serve the much-needed function of bringing the groups together so that each may come to appreciate better the views of the others.

2.3 REPORTING

Consistent with the policy for administration of University Centres, the Centre for the Study of Animal Welfare will report to the Office of Research. However, it will be the duty of the Director and the Steering Committee to ensure that the Centre functions in a manner that is independent of the special interests of any Department, College or sub-unit of the University, and of any outside body.

2.4 DEVELOPMENT

It is proposed that if the Centre is approved by Senate it should develop in a sequence of three phases:

Phase 1 (Year 1) Appointment of Steering Committee. Fund raising by the Steering Committee and other members of the University. Consultation with interest groups.

Phase 2 (Year 2) Search for Director, appointment and commencement. Establishment of Director's office and operating budget. Continued fund raising.

Phase 3 (Year 3) Appointment of Core Faculty. Establish Centre Operating Budget. Continued fund raising.

Phase 4 (Year 4) Centre fully operational. Continued fund raising.

2.5 BUDGETARY CONSIDERATIONS

It is proposed that the development of the Centre would only proceed if outside funds are obtained. Outside funds would be expected to constitute most of the Centre's finances, but the Centre would look to the University for some support in three ways:

i) an initial seed fund to enable the Fund Raising of Phase 1 to proceed efficiently,
ii) a commitment to provide the supporting costs of grants awarded by outside sponsors and,
iii) provision of a Faculty appointment for the Director.

The projected budget for the four phases of development are shown in Figure 2.

ACKNOWLEDGEMENTS

We are grateful for helpful comments on this proposal from the following: Dr. B. Segal, President; Dr. J. MacDonald, Vice President Academic; Dr. W. Marsh, Office of Research; Dr. F. L. McEwan, Dean of Ontario Agricultural College; Dr. B. Sells, Dean of College of Biological Science; Dr. N. O. Nielsen, Dean of Ontario Veterinary College; Dr. T. Settle, Department of Philosophy. We also acknowledge gratefully Dr. J. Mahone's Chairmanship of the Committee for the initial stages of drafting the proposal.

Appendix I

Courses currently offered at the University of Guelph dealing specifically with the subject of animal welfare, include

10-415 Principles of Farm Animal Care and Welfare
Department of Animal and Poultry Science

73-260 Introduction to Laboratory Animal Care and Management
Department of Pathology

74-361 Ethics in Agriculture
Department of Philosophy

93-320 Ethics in Veterinary Medicine
Department of Veterinary Medicine

73-670 Laboratory Animal Science
Department of Pathology

In addition, the Animal Care Committee and the Faculty of Graduate Studies offers an Animal Care Short Course for Graduate Students.

Animal Care Services and the Office of Research also offer short courses on the subject of animal welfare.

Many other courses across the Campus deal with aspects of animal welfare, including animal health, nutrition, behaviour and welfare of captive animals, etc. A complete listing of such courses may be found in the University of Guelph Undergraduate Calendar.

APPENDIX II

CENTRE FOR THE STUDY OF ANIMAL WELFARE

LIST OF INTERESTED FACULTY

BARKER, DR. K.
PATHOLOGY

Standards of care and maintenance of wild animals in captivity in zoos, farms, parks, commercial displays. Standards of research/manipulation involving free-living wildlife (amphibia, reptiles, wild birds and mammals).

BENN, DR. D.M.
ANIMAL CARE SERVICES

I was the staff veterinarian of the Central Animal Facility for 6 years and have been the Director, Animal Care Services for the past 2 years. In these capacities, I have been given the responsibility for ensuring the wellbeing of animals used in teaching and research at this University. I am also involved with the process for approving the use of animals in teaching and research and have played an active role in the University of Guelph Animal Care Committee. My interests in animal welfare include the administrative aspects of animal welfare as it pertains to large institutions such as ours as well as the need for further research endeavours into alternate husbandry practices and common laboratory practices.

BHATNAGAR, DR. M. K.
BIOMEDICAL SCIENCES

Reduction in the use of animals in toxicology.Development and validation of in vitro toxicology methods in avian species.

BROOKS, DR. R. J.
ZOOLOGY

Animal behavior is my speciality. Research on management and conservation, on methods of "control", and on dog behavioral problems. Extensive experience of animals in laboratory and field relevant to animal welfare.

BUTLER, DR. D. G.
CLINICAL STUDIES

I am particularly interested in being involved in scholarly research to assess the impact of close confinement and intensive systems for veal calf rearing on both productivity and animal well-being. Likewise, I am interested in evaluating the impact of loose housing of dairy cattle versus the stalls in overall animal productivity, health or well-being.

CHAPMAN, DR. H. W.
BIOMEDICAL SCIENCES

Use of animal models in laboratory teaching and development of suitable substitutes. Implications of behavioural research for the welfare of farm animals and of companion animals.

COCKSHUTT, DR. J. R.
CLINICAL STUDIES

Use of teaching animals in veterinary training and in particular the use of animals in surgical training programmes.

CONLON, DR. P. J.
BIOMEDICAL SCIENCES

The control of pain in experimental procedures by therapeutic and other measures.

CROY, DR. B. A.
BIOMEDICAL SCIENCES

Intermediate animal modelling in immunology and reproductive biology using xenografting to mice of immunodeficient genotypes. General Animal Care Committee Responsibilities.

DAVIS, DR. H.
PSYCHOLOGY

Higher order mental activities in animals.

DOWNEY, DR. R. S.
OVC DEAN'S OFFICE

I am interested in (i) the environmental ethic which involves the animal and its environment, (ii) the human animal bond and

(iii) the ethical and practical issues which relate to the use of animals in research and teaching.

DOWNIE, DR. H. G.
BIOMEDICAL SCIENCES

Regularization of experimental surgicalprocedures.Course development for experimental procedures using animals, for non-veterinary medically trained personnel (non-D.V.M. animal technicians).

DUNCAN, DR. I. J. H.
ANIMAL AND POULTRY SCIENCE

I have been carrying out research into various aspects of the welfare of farm animals (mainly poultry) for 24 years. Areas of particular interests have included: identifying behavioural and physiological indicators of states of suffering such as frustration, fear and pain; measuring the aversiveness of these states of suffering; measuring the motivation of animals to perform behaviour which appears to be inhibited by intensive husbandry systems; using these results to build models of motivation which will clarify the notion of "ethological needs"; investigating spacing behaviour; assessing the implications for welfare of the level of cognitive ability held by an animal; applying the results of all these investigations to improve welfare.

FERGUSON, DR. H. W.
PATHOLOGY

General aspects of fish health including farmed species, those kept as pets, and wild species.

GENTRY, DR. P.
BIOMEDICAL SCIENCES

My general interests in the field of animal welfare focus on two aspects. The first concerns the development of teaching materials, particularly in the area of laboratory exercises, which replace the use of live animals while enhancing the learning experience for the student. The second concern involves the development of alternative approaches to the use of live animals for research experiments.

HUNTER, DR. D. B.
PATHOLOGY

I am involved with the wildlife rehabilitation facility at O.V.C. and give numerous talks locally and nationally regarding wildlife rehabilitation. I work with commercial mink and fox and have been involved in drafting the code of practice for handling both mink and fox and I am presently a delegate on an international committee addressing the concerns of animal welfare in the fur industry.

HURNIK, DR. J. F.
ANIMAL AND POULTRY SCIENCE

Main area of study is the behaviour of farm animals. Research objectives focus on better understanding of behavioural symptoms of animal wellbeing (or animal suffering) and on de-velopment of production systems which provide high quality of farm animal life jointly with improved animal productivity (e.g. new housing system for laying hens, advance feeding and housing system for sows, mechanical nursing device for neonatal piglets). Initiated, with assistance of Dr. H. Lehman, Department of Philosophy, a course entitled "Principles of Farm Animal Care and Welfare". Drafted national code of practice for Poultry, Swine and Dairy Producers.

JOHNSON, DR. W.
POPULATION MEDICINE

It is unlikely that I will ever become a "core" faculty member, however I believe this is a very important effort and would like to show my support. It is important that the veterinary industry be a leader in this field and that animal production management systems be based on humane practices.

JOHNSTONE, DR. I. B.
BIOMEDICAL SCIENCES

Interested in promoting the use of animals in non-terminal experiments i.e. where the animal is used to provide a source of biological materials for experimentation. Also interest in the use in research of animals (including "pet animals") with naturally occurring disorders (i.e. "animal models" or clinical cases).

JULIAN, DR. R.
PATHOLOGY

Poultry welfare: housing, management, catching, trucking, processing. Birds as research animals. Poultry research. Pet and cage birds.

LAVIGNE, DR. D. M.
ZOOLOGY

Integrating (and attempting to understand) animal welfare interests as one component in discussions of complex (and controversial) wildlife issues such as the seal hunt, whaling and the trapping of fur bearers.

LEHMAN, DR. H.
PHILOSOPHY

What is animal welfare? How can we measure animal welfare? How can we weigh animal welfare in relation to other objectives? Should urban planners be required to make provision for theanimals displaced from their habitats? Under what circumstances is it acceptable to kill animals? Should scientific research, not directed at solving essential needs of humans, e.g., health problems, be restricted if it requires causing animal pain or death? How should we describe animal psychological states?

LUESCHER, DR. U. A.
POPULATION MEDICINE

Farm Animal Welfare and Farm Animal Behavior. Practical and research methods to evaluate farm animal welfare. Assessment of housing/production systems with regards to animal welfare. Welfare 'and performance: philosophical aspects and cost-benefit analysis of improving welfare. Animal welfare legislation, codes of practice, industry standards. Philo-sophical issues related to the use of animals for food.

McDONELL, DR. W. S.
CLINICAL STUDIES

Perioperative pain control for experiments needing same. Perioperative pain control for (research) laboratory animals (consultation).

McKEOWN, DR. D.
POPULATION MEDICINE

Involved in studies related to stereotypes of dogs, cats, and horses as well as other stress related behaviors due to environment and management. Currently involved with Bill 129 - The Zoo Licensing Act.

McMURTRY, DR. J.
PHILOSOPHY

Suffering of animals under experimental conditions. Experimentation with animals for teaching purposes or for non-life-saving commercial product development. Mass production and slaughter of animals for consumption where more efficient systems of protein production and consumption are available.

MEEK, DR. A. H.
OVC DEAN'S OFFICE

I am currently conducting research in the area of the human animal bond. The work involves an investigation of the degree of bonding between grade 5 children and their pets, and its relationship to their development.

MICHALOS, DR. A. C.
PHILOSOPHY

The only animals whose welfare I have studied are humans, but I edit Social Indicators Research and have published several articles and books on the quality of life, happiness, satisfaction and general well-being of people.

MITSCHERLING, DR. J. D.
PHILOSOPHY

General interest in animal welfare.

MILLER, DR. R. B.
PATHOLOGY

Alternatives to the use of animals in research and teaching.

MORRISON, DR. W. D.
ANIMAL AND POULTRY SCIENCE

My teaching relates to environmental

physiology. My research is in the general area of the environment including heat stress, air-borne contaminants, selection ofan environmental temperature by animal, etc. I work with pigs and chickens.

MUCKLE, DR. C. A.
VET. MICRO AND IMMUNOLOGY

Racing of young thoroughbreds/ standardbreds, housing of poultry, housing of swine, pet welfare standards.

PASCOE, DR. P.
CLINICAL STUDIES

Interested in the use of analgesics in animals in particular the opiates and alpha-2 agonists. As an anesthetist I am interested in the prevention of animal suffering and have in the past worked on 'boar bashing' in Ontario and on the humaneness of electroimmobilization.

PORTER, DR. D. G.
BIOMEDICAL SCIENCES

Philosophy of our relationship with non-human animals. Development of basic biomedical research using non-, or minimally, invasive methods. Ethical aspects of genetic engineering. Teaching techniques which minimize animal use (e.g. plastination).

RONALD, DR. K.
ZOOLOGY

Humane handling of captive and free living animals.

RUSE, DR. M.
PHILOSOPHY

Ethical issues in human-animal relationships.

SETTLE, DR. T.
PHILOSOPHY

I believe two things. First, we do not, in general, in Canada, treat all animals absolutely as we should. Secondly, we have not yet determined with enough precision or with general acceptance, for all species, just <u>how</u> we should. I am deeply interested in animal welfare. Nine species share the life of our farm, most being raised by us primarily for food. My academic interest, sparked, no doubt, by my sense of - how should we put it - fellow-creaturehood with them, is in determining how animals should be treated and in trying to describe the grounds for the standards we determine.

SMOOK, DR. R.
PHILOSOPHY

I have no special expertise. I just dislike unnecessary animal suffering and would like to do what I can to prevent it.

SUMNER-SMITH, DR. G.
CLINICAL STUDIES

Fracture healing, joint replacement, canine and feline dentistry, bone density.

SUMMERLEE, DR. A. J. S.
BIOMEDICAL SCIENCES

Uses of experimental animals with particular emphasis on the use of unanaesthetized, unrestrained animals for study of the nervous system. Development of alternative methods for teaching gross veterinary anatomy by use of plastinated materials.

THOMAS, DR. V. G.
ZOOLOGY

The fur trapping industry: trapping techniques and the scientific criteria for assessing harvest levels in wild populations. Intensive rearing facilities versus free range conditions of animal rearing in low-input, agricultural systems.

WALTNER-TOEWS, DR. D.
POPULATION MEDICINE

Animal welfare within the larger ecological context - e.g. wildlife, species extinction, hunters, eating meat, etc. Animal welfare in cultural context - e.g. is crowding <u>per se</u> wrong? Does that mean the whole island of Java, or Bangladesh, is <u>wrong</u>? Or is the issue one other than crowding?